

# MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

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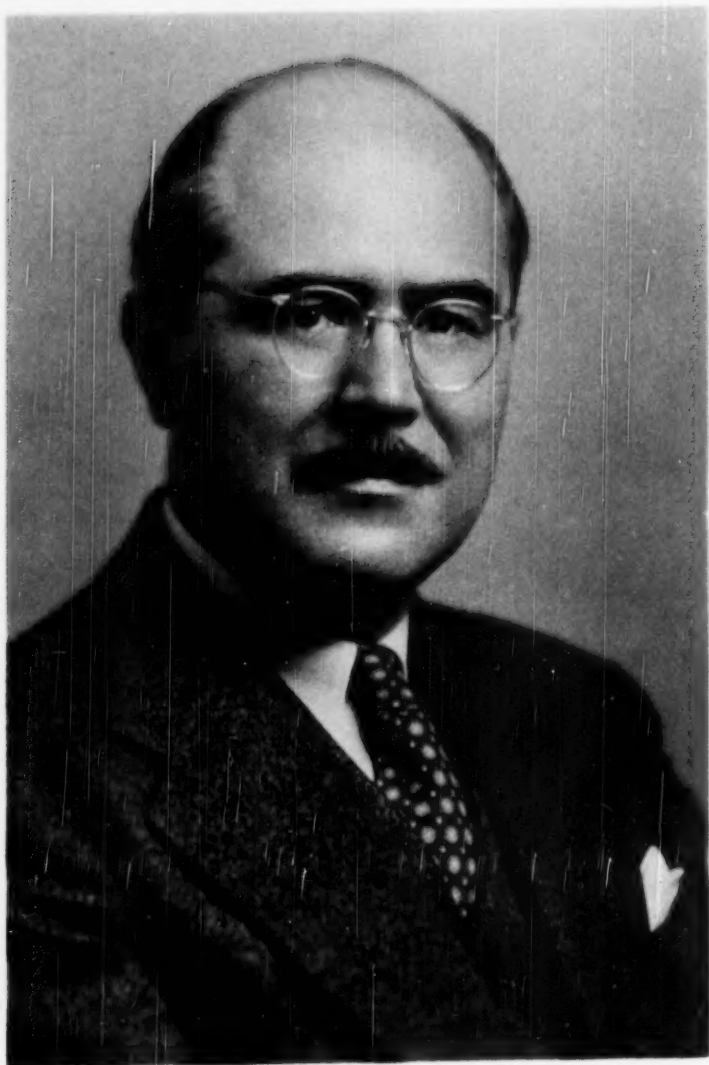
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# MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XLII NOVEMBER-DECEMBER, 1950

No. 6

## THE EXPANDING HORIZONS OF MYCOLOGY<sup>1, 2</sup>

F. K. SPARROW

In the history of any science certain periods of intense and productive work are easily discernible. Sometimes these are the result of the enthusiasm of an unusually successful teacher or keen research man. Sometimes they come about because of the discovery of organisms ideally suited for certain types of experimentation. Sometimes new tools for the investigation of special problems are discovered. Sometimes because of pressing national necessity, fields of investigation quickly open up that were undreamed of for the immediate future. Sometimes all of these elements combine to produce a relatively rapid reorientation and fresh approach to the science.

The field of mycology, for example, has been given a new impetus and drive in the United States in recent years by some or all of these elements. It would be informative and no doubt interesting to assemble data from our journals of the past 40 years on the changing emphasis in fields of investigations on fungi. It seems sufficient here, however, to put these in general terms.

For many years research in mycology has been primarily morphological and taxonomic, and it has been fruitful work, indeed. To be sure, there has always been an interest in the physiological, genetical, and general "experimental" avenues of approach, but

<sup>1</sup> Presidential Address, Mycological Society of America, New York, 1949.

<sup>2</sup> Contribution No. 911 from the Botany Department, University of Michigan.

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it has not been great or sustained and has often dealt with specific isolated processes or products. There can be little question but that the excellent fundamental groundwork done in earlier years in the taxonomy and morphology of fungi has been a potent influence in making possible the recent development and expansion almost by leaps and bounds of new fields of investigation.

We are at the moment watching on the sidelines, as it were, a beautiful and what will unquestionably come to be regarded as a classical example of the use of a fungus in unraveling the complicated skein of biochemical syntheses.

The success of the current work on the biochemical-genetics of *Neurospora*, presents to the speculative, general mycologist much food for thought. Here, because of the excellent morphological groundwork laid by Shear, B. O. Dodge and others, a team of investigators, chiefly interested in genetics and biochemistry, and utilizing genetical data, accumulated primarily by Dodge and Lindegren, has underlined a fresh approach to mycological study, the biochemical-genetical attack on fungus metabolism.

To be sure, a great measure of their success is due, as they readily admit, to the special qualities of the fungus itself, and this is a point of more than passing interest. Too long, perhaps, have some of us regarded the activities of our special pets with an unappreciative eye. We must come to a greater realization of the fact that among the fungi are organisms ideally suited for the study of fundamental, universal physiological processes. In *Neurospora*, for example, its simple nutritional requirements and high rate of growth favor biochemical studies. The fact that it is heterothallic and haploid favors a clear interpretation of genetical data since no problems of dominance and recessiveness occur. The linear arrangement of the ascospores in the ascus and their origin facilitates further precise genetical analysis and, since all the ascospores ordinarily are viable and give rise to colonies, no meiotic products are lost. A further favorable feature is the rapidity with which the whole life cycle is completed. The problems of both genetics and biochemistry can be reduced to unusually simple terms.

I need not repeat the fruitful and stimulating results of this particular series of investigations nor emphasize their fundamental

biological significance. There is given us here still another line of evidence which points to the universality among living things of basic phenomena, whether they be reduction division or amino acid requirements.

It would be intriguing to speculate on the wider application of the *Neurospora* work. Research in the complex problems of human nutrition and metabolism will beyond question benefit greatly. In our own area of study, biochemical mutants artificially produced might well in the future give us clues as to the origin of parasitism among the fungi.

In the Aquatic Phycomycetes, there exists a field ripe for a new approach. We have up to now been primarily concerned in discovering just what forms are present in nature and in learning something of their morphology, reproductive processes, habits, etc. Very definitely, this work has been in the classical tradition of the older mycology. The prediction I made two decades ago that there must exist an enormous microscopic phycomycetous flora, only a few scattered examples of which we then knew, has been abundantly confirmed. And we have by no means even now exhausted the field of pure discovery, as any attentive reader of the *American Journal of Botany*, *Mycologia* or the *Transactions of the British Mycological Society* will readily confirm! A modern young Linnaeus can, without too much difficulty, with suitable procedures, ferret out whole hitherto unknown or little known groups.

Something more fascinating than the discovery of new species, however, has emerged from these researches on Aquatic Phycomycetes—something which presents to experimental mycology a fertile field for investigation. We read after the routine technical descriptions of new species of chytrids a somewhat bizarre and almost incredible list of substrata: "On chitinous exuviae of aquatic insects," "cellophane," "onion skin," "pollen grains," "green algae," "shrimp skeleton," "snake skin," etc. In other words it is now evident that even in this single group of the Phycomycetes we have an aggregation of microorganisms with vast capacities for the production of diverse enzyme systems and a general metabolic dexterity of a high degree. Further, when we read that a single, well-defined morphological species such as *Catenaria anguillulae* can utilize such a range of substrata as eel

worms, liverfluke eggs, algae, snake skin, mosses, ferns and dead angiospermous tissue we appreciate old Haller's well-known and oft-quoted characterization of fungi as "... a mutable and treacherous tribe. . . ."

We are only beginning to culture these extraordinary chytrids with any degree of facility. With the advent of rapid, sure methods of obtaining and maintaining them in pure culture, a broad field for physiological investigations will open up which will beyond doubt be one of unusual fruitfulness in both the areas of biochemistry and general nutrition.

It is heartening to see that a successful start has been made on basic physiological problems of Lower Phycomycetes by using modern physiological methods and tools. From what little we now know, their morphological diversity is only equalled by their capacity to derive nutriment from a wide variety of materials.

Research on the genetics of the Aquatic Phycomycetes, however, has been limited by various considerations, not the least of which has been the difficulty of getting some of them into pure culture and maintaining them. Furthermore, before any extensive work can be pursued along genetical lines, methods must be developed for securing ready and abundant germination of the diploid phase by the production of uninucleate zoospores. This has been done with a high degree of success primarily in the Blastocladales and it is in the genus *Allomyces* of that order that basic genetical work has been initiated and where it will no doubt be most quickly developed. Here, male and female gametangia are readily separated and interspecific hybrids can be produced. Here, also, the resting spore, or its equivalent, can be subjected to outside physical and chemical forces during meiosis, and artificially produced mutants obtained. Furthermore, since the fungus is easily cultured, nutritional studies of a fundamental nature can readily be undertaken. Other features such as its coenocytic nature, rapid rate of growth, simplicity of structure, fast life cycle, etc. make it equal, to my mind, to *Neurospora* for biochemical and biophysical studies.

We have the tools and we have the fungi. We can look forward, now, I believe, to a new era of investigation on Lower Phycomycetes by persons trained in modern physiological methods, using modern tools of attack.

Returning to a more general consideration of mycology, we see on all sides new developments which project the study of fungi into areas little or not at all touched upon in the past. I can mention here only a few of these. There are many others, as for example the work on variability, studies on the production of fats by fungi, the use of fungi in biological assays, etc.

Under the impetus of our recent national emergency, mycology has suddenly expanded into the arena of national defense. The establishment of the Deterioration Laboratories by the Army Quartermaster Corps, organized and guided by the first President of our Society, William H. Weston, proved of great value in solving the immediate problems of materiel deterioration and in meeting new ones. War brought to the fore this somewhat neglected aspect of mycology. There was an almost belated appreciation of the rôle of fungi in the destruction of fabrics, leather, delicate instruments of essential communication, and even lenses. All or nearly all of these deleterious activities of fungi were known beforehand. However, the tremendous scale on which this deterioration of essential war materials took place in a global war, especially as waged in the tropics, pointed up the problem and convinced even the most skeptical of the need for prompt and efficient teamwork.

The work of the Biological Laboratories of the Quartermaster General Laboratories is being continued. This organization embraces laboratories of Microbiology, Biochemistry, Physiology and Biological Testing, all of which are concerned with various aspects of fungus metabolism in relation to the deterioration of military and industrial materials. Many hundreds of cultures of fungi isolated from deteriorating military equipment from all over the world are maintained in active condition both at Philadelphia and at the Farlow Herbarium at Cambridge, Mass., where part of the project is placed. Thousands of others are kept viable but in an inactive state for possible future study. Each fungus in the active collection will eventually be studied in all aspects of its relation to deterioration. Especially significant species which lend themselves to such treatment will be used in biochemical investigations of various processes of deterioration. The results of these continuing

studies will be of unquestioned value to the military and to private industry.

The proven value of mycological investigations in solving problems of deterioration in vital naval equipment, has resulted in the formation of a section of Moisture and Fungus Proofing in the Bureau of Ordnance of the Navy Department. In this organization the mycologist has such varied duties as: development of research programs in moisture- and fungus-proofing, both within the Navy and in industrial and research institutions; determination of the extent of damage by fungi and moisture on shipboard; writing specifications for moisture and fungus proof varnishes, etc.; devising tests for materials; consultation with naval establishments and contractor groups; liaison and committee work with groups both within and outside the Navy. The activities of this section demonstrate not only the present importance of mycological work in vital defense but the diversity of duties the modern "practising mycologist" may be called upon to perform.

Let us turn for a moment to another aspect of mycology. It seems hardly necessary today to call special attention to the field of antibiotics. Within a short space of time a new area of fungus study and a giant industry have been developed. This lusty young industry has in many cases been both guided and developed by mycologists who were specialists in fields far removed from their present one. They left their former research interests and with admirable courage turned their energies towards developing and expanding the commercial aspects as well as the purely scientific ones of a new field of mycology, a field of our science of vital importance to every human being. It is plain to see that as a result mainly of their efforts, fungi have definitely entered big business, and they seem there to stay.

As we find out more and more about the workings of these powerful therapeutic agents, we wonder if there may not be in nature many other, undiscovered, strains not only of *Penicillium*, etc., but of other fungi, which might yield products to fulfill diverse clinical needs. Perhaps we have just scratched the surface of this phase of mold metabolism and perhaps there await us, either in nature or in experimentally produced strains, organisms capable

of yielding a vast array of substances of both medical and general commercial importance.

Along with this resurgence in the commercial use of molds there has run a parallel expansion of what has been called "fermentation engineering." The growing of large quantities of fungi under sterile, controlled conditions and the refining of their products have brought forth new problems in plant construction, new types of apparatus, machinery, etc. This, too, is a part of the expanding mycological picture, for these engineers are dealing with problems of fungus growth and metabolism on a large scale.

One cannot omit mention at this point of the part played by the Northern Regional Research Laboratory in the development of improved methods of penicillin production. The wartime collaborative efforts of this organization with other centers of research on this problem not only resulted in notable increases in the yield of this valuable drug by way of high-yielding strains, etc., but also materially assisted in putting penicillin production on a sound commercial basis. Wartime governmental agencies considered the penicillin program second only in importance to the perfection of the atom bomb. This same N.R.R.L. group also gave effective service during the war to the studies on deterioration being carried on by the Quartermaster Corps. The wisdom of seeing to it that the Regional Research Laboratories were staffed with able mycologists and microbiologists has been of immeasurable service to the country. At present, the Culture Collection of the Fermentation Division of the Northern Regional Research Laboratory carries over 5000 species and strains of filamentous fungi, yeasts, bacteria and actinomycetes. These are maintained for a dual purpose: to supply microorganisms for their own investigations on fermentations and to supply research laboratories both here and abroad with cultures of significance in fermentations and other fields of microbiology. One of the most important aspects of the collection is the maintenance of multiple strains of particular species which are of known or potential value in important fermentation processes.

We might turn to yet another aspect of our science—Medical Mycology. This area has been gradually assuming greater and greater importance among active practitioners and clinicians and

to some degree in medical education. Possibly this has come about because a few well-qualified mycologists and physicians have had a continuing interest in the field. Working closely with those familiar with the clinical aspects of fungus diseases in man they have accomplished a great deal. We can look forward, I believe, to an increasing demand from medical centers for persons trained in basic mycology who will further specialize in medical mycology. How much can be done in an already crowded medical college curriculum towards increasing the time devoted to a study of fungi is problematic.

We might mention at this point the establishment by the government, under the National Institutes of Health, of an organization devoted primarily to the study of large fundamental problems of mycoses in man. This group undertakes field studies on diseases suspected to be of fungus origin. It also carries on extensive surveys of pathogens peculiar to certain areas, with special emphasis on such problems as possible wild hosts. Since most of the fungi causing systemic and fatal mycoses in man are not transmitted to persons in contact with the patient, the epidemiology and the source of the infection become matters of greatest importance. The positive demonstration by these workers of the occurrence of coccidioidomycosis and histoplasmosis in wild rodents goes far towards explaining the peculiar geographic distribution of some diseases. Not only does this laboratory provide a diagnostic and identification service as a practical aid to physicians, hospitals and state health laboratories, but it also carries on work on the improvement in diagnostic methods and the dissemination of information about them. It also pursues studies on skin-testing as a diagnostic method and at present is working on serologic methods of diagnosis and studies of the nutritional requirements and responses of fungi with special reference to serologic methods. Studies of fungicides have also been undertaken. An expansion of this important aspect of mycology, touching as it does on the public health and welfare, seems eminently desirable and would serve the country well.

Other agencies which have contributed to this phase of mycology could be mentioned, for example, certain pharmaceutical houses, but this seems sufficient to illustrate that here, as elsewhere, mycology is on the march and is penetrating more and more extensively

and effectively into what were once regarded as peripheral areas of the science.

To my mind, our organization stands at a critical point in its history in relation to these newer developments in mycology. We should give full recognition to the fact that there has arisen what might be termed a "new mycology," which merits our fullest possible cooperation. Unquestionably, morphological and taxonomic work will always be a basic and integral part of our science. This new mycology, however, is a logical outgrowth not only of the experimental trend of biological science in general, but of the times through which we are passing.

What can we do? Few of us, indeed, can pick up the long-faded strands of our early training in chemistry and become biochemists of fungi. More of us, perhaps, can put our hand to other, experimental, aspects of the groups with which we are familiar and make some use of the modern tools of experimentation which have been made available to us. All of us who are teachers can assist in the development of this phase of mycology by acquainting our students with the many opportunities for research in the experimental aspects of the subject and by seeing to it that they are properly prepared in the basic sciences to pursue such researches on fungi.

It seems to me that at this time certain features of the Society should be discussed and decisions made. We ought to have within the framework of the Mycological Society provision for those interested in these new areas of mycology. For example, there might well be a section concerned with research and problems in "mycological" or "microbiological engineering." This expanding field lies, to be sure, on the borderline of our science, yet it is concerned with fungi. There could be formed with profit to all a section of the Society dealing with problems arising from the industrial uses of fungi. Those interested in the physiology of fungi are most certainly people who should be members of the Society. Much can be gained by mutual contact, and the views of all will be broadened. Thinking along these same lines, we might well include the field of deterioration and preservation. Here is another group dealing with the applied aspects of mycology. To be sure, many of their problems are problems of chemistry, manufac-

ture, etc., but they do center around fungi. Amateur groups should be encouraged to affiliate with us. To form independent societies concerned with individual facets of mycology results, it seems to me, in a loss to all. The new society centers its interests around a small field and can easily lose contact with the broader aspects of the science. The parent group, in turn, loses the catholicity of interests which an organization of national stature should possess.

It might be pertinent here to emphasize that the Mycological Society of America is not solely a society for the study of fungus taxonomy. That it has seemed like the latter to some, can be traced in part to the fact that the founders of the Society were in large part interested in taxonomy and this was naturally reflected in the activities and publications of the Society.

Let me say, in conclusion, that the Mycological Society, I feel sure, is interested as it always has been, in *all* phases of fungi, and that as the horizons of mycology continue to expand, the Society will keep pace with them.

## THE RELATION OF NUTRITION TO THE GROWTH AND MORPHOLOGY OF TRICHOPHYTON FAVIFORME

LUCILLE K. GEORG \*

(WITH 6 FIGURES)

The large-spored ectothrix trichophytions of animal origin were described by Sabouraud in 1893 (1) as *Trichophyton faviforme* or "trichophytions of faviform culture" because of the resemblance of their colonies to those of *Trichophyton Schoenleini*, the causative agent of the disease favus. These fungi were extremely slow to develop on the sugar-peptone media of Sabouraud and, even after 3 or 4 weeks of growth, produced button-like colonies only 10 to 15 mm. in diameter. The colonies were usually raised and often acuminate (occasionally flat), with an irregularly folded surface. They were tough and leathery in texture. The surface was glabrous and at times moist, with a small amount of white powder or velvety surface growth developing on older colonies. Microscopic examination revealed a highly irregular, twisted, degenerate type of mycelium with many chlamydospores. Microconidia and macroconidia, the characteristic spores of the genus *Trichophyton*, were not observed. Although their cultural appearance resembled that of *T. Schoenleini*, Sabouraud separated these fungi from the latter because of their common occurrence on cattle and horses, their ectothrix appearance clinically, and their association with circinate or suppurative lesions typical of ringworm rather than of favus.

E. Bodin (2), who studied the culture described by Sabouraud as *Trichophyton faviforme*, isolated several similar organisms from lesions on horses, a donkey, and a calf. In addition, Bodin described a strain which he at first called "Trichophyton faviforme

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du veau" but later named *T. verrucosum* (1902). His description was as follows: "On glucose agar the culture forms a little cake in a month's time. Part is submerged in the agar, and the surface is irregular, verrucose, and grey."

In 1908, Sabouraud (3) described two species in this group. One which he called *Trichophyton ochraceum* corresponded closely to the *T. verrucosum* of Bodin except that on maltose agar it formed a yellow ochre pigment. The second species, *T. album*, was difficult to distinguish from *T. Schoenleini*. It formed a glabrous, acuminate colony with regularly folded sloping sides. Some colonies were umbilicate and resembled a miniature volcano. Sabouraud stated that it was extremely slow-growing and poorly developed in comparison with cultures of *T. Schoenleini*. He found that primary cultures had to be placed in an incubator at 37° C. to obtain best development. After continued cultivation, the organism occasionally developed a fine white powder, or an irregularly distributed short white down over the surface of the colony. In 1910, Sabouraud (3) described a fourth species, *T. discoides*, which he stated was similar to *T. album* except that, in culture, it formed an almost perfect disc with a flat surface and often a central knob. Some colonies had a slight brownish color and a moist appearance while others developed a fine, short white velvety growth over the entire surface.

Many workers have studied the organisms of the so-called "faviform group" in an attempt to obtain spores which would identify them more clearly as members of the genus *Trichophyton*. The introduction of the "natural media of polysaccharide base" by Langeron and Milochevitch (4) has aided this study greatly. On these media, which consisted of whole grains of wheat, barley, corn, and oats, the fungi developed quite rapidly, producing an appearance entirely different from that of the glabrous colonies obtained on the sugar-peptone media of Sabouraud. A white powdery or even fluffy growth spread over the surface of the grains and microscopic examination revealed microconidia, characteristically borne singly along the hyphae (en thyrsé) and terminally in clusters (en grappe). Rudimentary spirals and one abortive macroconidium typical of the *Trichophyton* genus also were demonstrated.

In 1934, Lebasque (5) carefully reviewed the "faviform group"

and was able by the use of the polysaccharide media to complete morphological studies of the known species as well as of three new species. Lebasque accepted the following as valid members of the genus *Trichophyton*: *T. verrucosum* (Bodin, 1902), flat, with verrucose surface and a grey color, *T. ochraceum* (Sabouraud, 1908), flat and verrucose, but with an ochraceous pigment; *T. album* (Sabouraud, 1908), heaped and folded, with a waxy appearance; *T. discoides* (Sabouraud, 1910), disc shaped with a small central knob; and *T. equinum* (Geddoelst, 1902), and *T. caballinum* (Neveu-Lemaire, 1921), two strains isolated regularly from horses, which showed a white, downy to fluffy surface but were otherwise similar to other strains in this group in their lack of ability to produce spores on the sugar-peptone media. His three new species were *T. bulbosum*, *T. villosum*, and *T. papillosum*.

Since many of these species had been designated solely on the basis of colony size and shape, degree of downiness, and pigment production, characteristics which are highly variable among dermatophytes, it is probable that several are actually synonymous. It seemed important to study a series of recently isolated strains in this group as well as cultures from stock collections in order to learn the range of variation which could be expected in a single strain.

Furthermore, since it has been shown that under certain conditions these fungi can grow rapidly and produce spores, it was hoped that more comprehensive studies on their nutritional requirements would establish their characteristic types of growth and spore production and thus furnish a firmer basis for their identification.

#### EXPERIMENTAL

##### *Part I. Cultural Studies of Recently Isolated Strains of T. faviforme*

The strains studied were isolated from cases of ringworm which had appeared among a group of farmers and their families in central Pennsylvania. In most cases large-spored, ectothrix involvement of the hairs had been observed, and the lesions, which occurred most frequently on the face, neck and scalp, were of a deep suppurative type. In all instances there was a history of

recent contact with cattle, and in several cases similar cultures were isolated from ringworm lesions on these animals. The clinical course and treatment of a series of 23 cases have been described in a previous publication (6). It was pointed out that it was occasionally difficult to isolate the fungi from these lesions using Sabouraud's dextrose agar and incubating at room temperature. However, by the use of a heart infusion-tryptose agar (Blood Agar Base, Difco) and incubation at 37° C. the chances for successful isolation were much improved. Although the fungi developed rapidly on this medium, growth was enhanced further when 0.1 mg. thiamine was added to 100 ml. of the melted partially cooled agar. On heart infusion-tryptose agar with added thiamine, the colonies were 30 to 40 mm. in diameter at the end of two weeks. They were often heaped and folded and covered with considerable surface growth which appeared as a fine powder or an erect white down. Microscopic examination of such colonies revealed a regular, flexuous mycelium with only occasional chlamydospores. The aerial growth contained large numbers of microconidia, and in several strains characteristic macroconidia were found as well.

#### *A. Growth on Sabouraud's dextrose agar*

On Sabouraud's dextrose agar, the fungi grew slowly and produced small, glabrous colonies resembling the species described by Sabouraud as *T. ochraceum*, *T. album* and *T. discoides* (FIG. 1, a, b, c). Microscopically only irregular mycelium and chlamydospores were observed (FIG. 2a). With frequent subculturing the strains could be maintained on Sabouraud's dextrose agar, but the colonial form was not stable. It was decided to study a series of single spore isolates in order to observe the possible range of variation within each strain.

Eight strains showing various colonial forms were selected for study. Spore suspensions were obtained from growth on the heart infusion-tryptose-thiamine medium described above, and six microconidia were selected from each strain according to the method described by Georg (7). The monospore cultures were studied over a period of several months. Some of the strains were quite stable and retained their original colonial form even after several

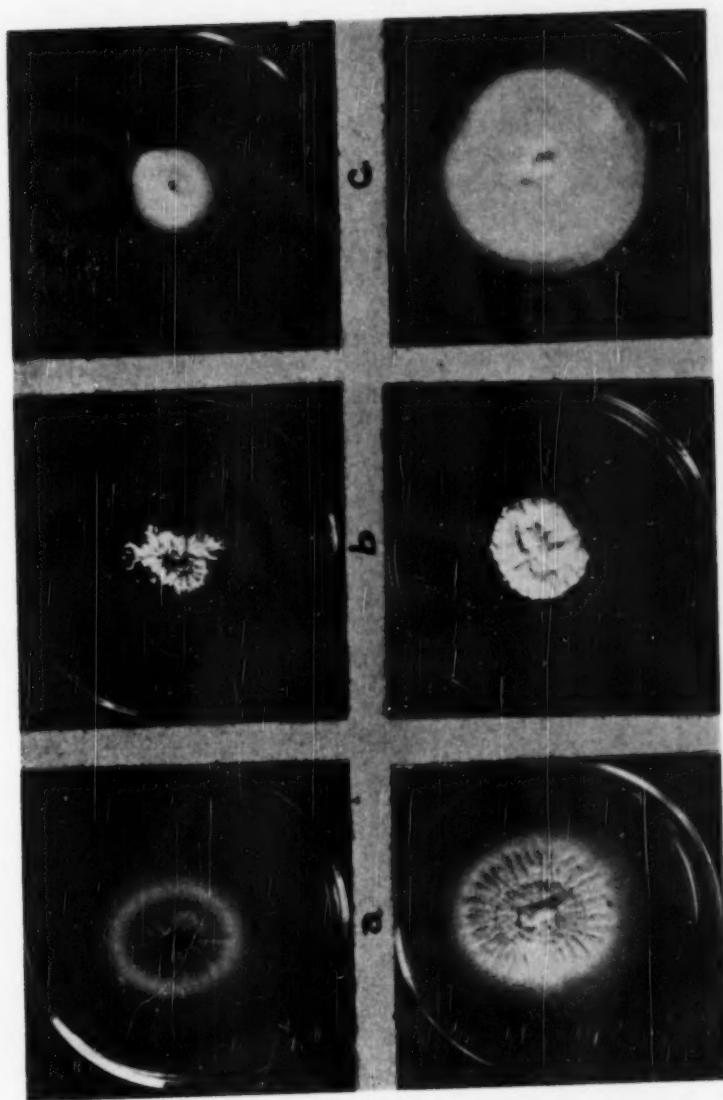


FIG. 1. a, b, c, *T. faviforme* (varieties *ochraceum*, *album* and *discoideus*) on Sabouraud's dextrose agar; d, e, f, Same cultures on trypticase-dextrose-thiamine agar.

transplants on Sabouraud's dextrose agar, but the large majority were variable, producing a wide range of colony forms. Figure 3 indicates some of the variants obtained from a single spore strain. Not only were there differences in shape and topography of the colonies, but also in the presence or absence of aerial growth and of pigment.

The possibility of variation in these organisms was first suggested by Cazalbou in 1913 (8) when he described *Trichophyton singulare*, a new species which had two cultural states, one glabrous and cerebriform, the other downy, flat, and disc shaped. The two forms were found to be reversible. In 1938, Gammel and Work (9) described a case of ringworm contracted from cattle and caused by a fungus which they called *Trichophyton album* var. *singulare*. After several transplants on a liver infusion agar, this organism produced two distinct types of growth, a cerebriform and a discoid.

These findings as well as our data obtained with single spore cultures seem to indicate that *Trichophyton album*, *T. discoides*, and *T. ochraceum* are not specifically distinct. It is suggested that the name *Trichophyton faviforme* be retained for the variants in this group. *T. faviforme* was the first designation employed by Sabouraud (1) and also by Bodin (2). It has had considerable usage, and is more generally descriptive of these organisms than names which represent highly variable colonial characteristics. The colony types may be designated as varieties: *album*, *discoides*, *ochraceum*, etc.

#### *B. Growth on whole grains*

The eight monospore strains of *Trichophyton faviforme* were planted on moist rice and barley grains. All the strains grew well, covering the grains with a felt-like mat. By the tenth day, most of the cultures had formed an erect aerial mycelium which extended beyond the kernels. A few of the strains produced only a fine, white, powdery growth at first but by the twenty-fifth day some down was visible here also. Microscopic examination showed regular, branching, flexuous hyphae with occasional intercalary and terminal chlamydospores. The aerial growth contained numerous microconidia scattered along the mycelium, as well as in

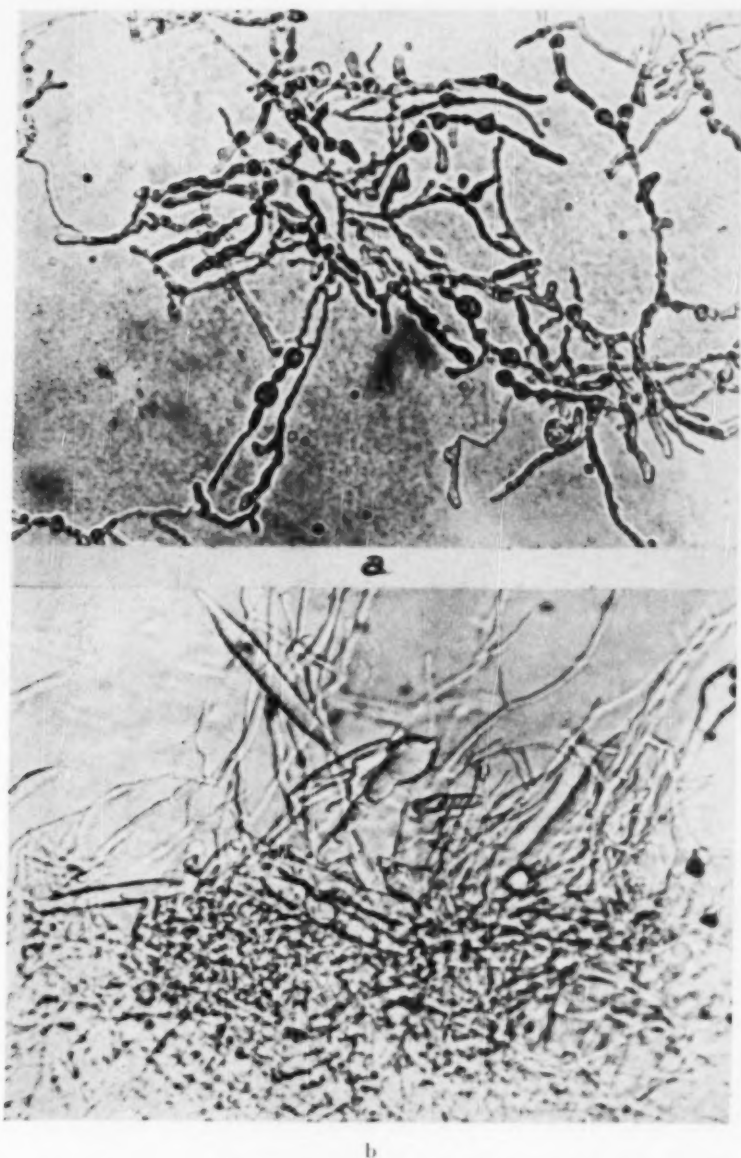


FIG. 2. *a*, Microscopic appearance of *T. faviforme* from growth on Sabouraud's dextrose agar; *b*, The same culture from growth on heart infusion-tryptose-thiamine agar.

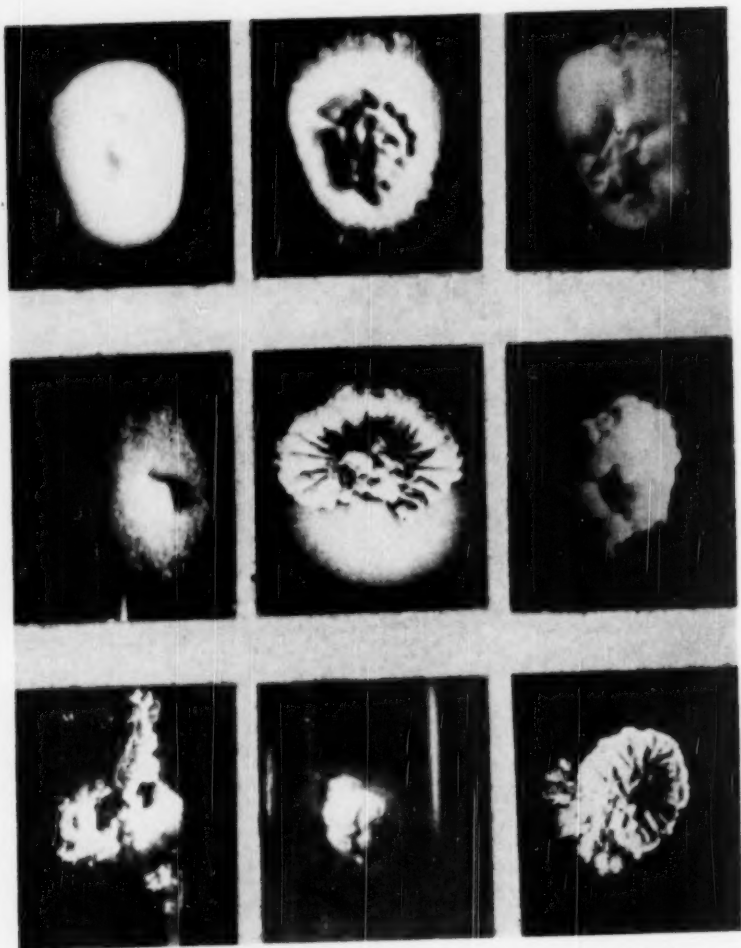


FIG. 3. Variation in colony form in a single spore strain of *T. faviforme*—Sabouraud's dextrose agar.

terminal tree-like clusters (FIG. 4a & b). These spores broke off squarely and easily from the point of their perpendicular attachment and were generally rather slender and elongate with considerable variation, measuring  $1.5-2 \times 3-4 \mu$ .

Macroconidia were found also in the downy to fluffy aerial mycelium appearing from the tenth to thirteenth day. These were thin walled,  $20-50 \times 5-8 \mu$ , with 1-7 septa, occurring singly either laterally or terminally. Some were short and bulbous, others had

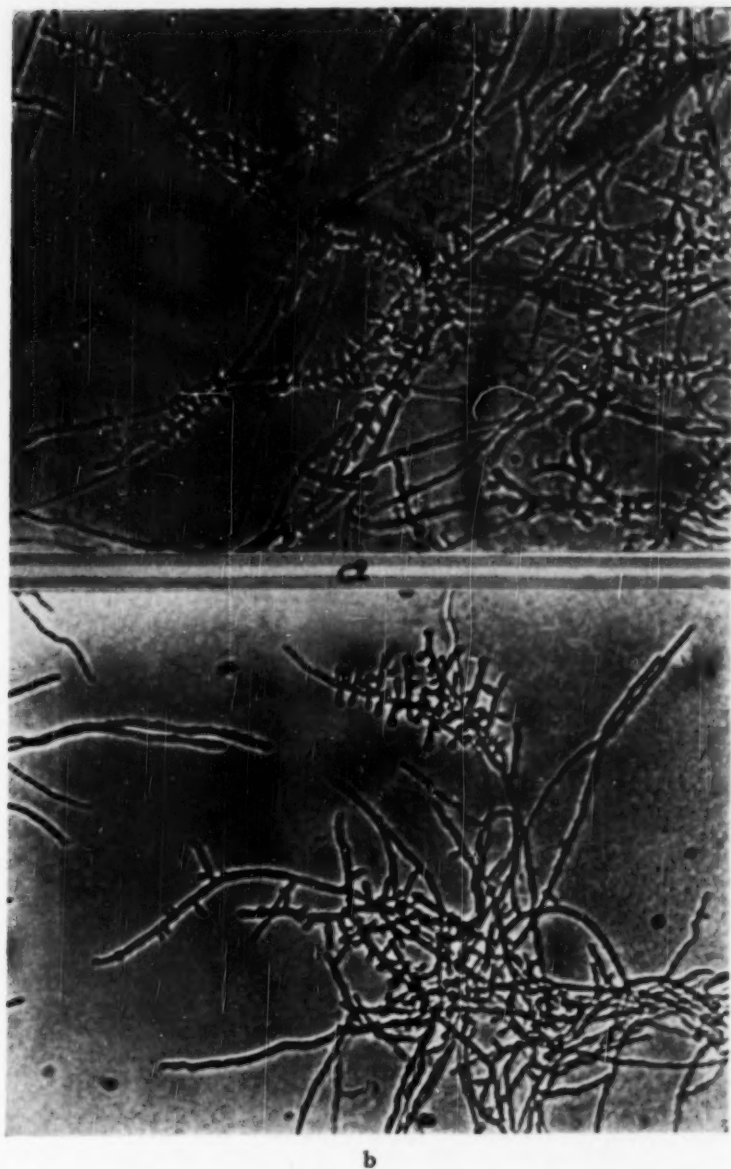


FIG. 4. *a*, Microconidia scattered along the mycelium from growth on rice grains; *b*, Microconidia in terminal clusters from growth on rice grains.

long pointed ends, and still others were irregularly swollen and distorted (FIG. 2b).

### C. Growth on enriched media

Growth was stimulated by both yeast extract and liver extract, the amount of aerial mycelium produced depending upon the strain as well as on the quantity of extract available. On Sabouraud's dextrose medium to which had been added 0.1 per cent of either extract, all strains grew rapidly, producing thick, compact colonies with a heavy, white, powdery, or occasionally white, fluffy, surface growth. Microconidia were numerous, and macroconidia appeared in some strains.

The stimulatory effects of Bacto-tryptose, beef heart infusion, and citrated human blood were studied in view of the fact that in the isolation work, blood agar plates had been used and found to be superior to Sabouraud's dextrose or maltose agar (6). It was thought at first that the whole blood in this medium was the stimulating factor. However, no difference was detectable in the amounts of growth on Blood Agar Base with and without the addition of whole blood. Both Bacto-tryptose and beef heart infusion were found to produce a stimulating effect when added to Sabouraud's dextrose agar in amounts comparable to those found in the Blood Agar Base. Trypticase, an enzymatic hydrolysate of casein (Baltimore Biological Co.), was studied also in an effort to find a practical, easily reproducible medium for routine isolation of and spore production by these strains. A one per cent trypticase-dextrose-thiamine agar was found to be satisfactory. On this medium there developed rapidly growing colonies with a white, powdery, surface growth or with a short, erect, white, aerial down bearing numerous microconidia and, in some strains, macroconidia (FIGS. d, e, & f).

The following media are recommended for isolation and production of rapidly growing, sporulating cultures of *T. faviforme*:

#### 1. Heart infusion-tryptose-thiamine agar

Beef heart, infusion from	500 g.
Bacto-tryptose	10 g.
Sodium chloride	5 g.
Bacto-agar	15 g.
Thiamine hydrochloride	10 mg.
Dist. water	1000 ml.

The ingredients are dissolved in the water, the pH adjusted to 6.8, and the medium autoclaved at 121° C. for 10 minutes.

This medium is similar to Difco Blood Agar Base, except for the thiamine. It may be prepared by adding thiamine to the dehydrated product.

2. Trypticase-dextrose-thiamine agar

Trypticase (Baltimore Biol. Co.)	10 g.
Dextrose	40 g.
Thiamine hydrochloride	10 mg.
Agar	20 g.
Dist. water	1000 ml.

The ingredients are dissolved in the water, the pH adjusted to 6.8, and the medium autoclaved at 121° C. for 10 min.

Since both of the above media will support bacterial growth, it is recommended that penicillin, 20 units per ml., and streptomycin, 40 units per ml., be added to the melted, partially cooled agar before pouring into tubes or plates.

For primary isolation, all tubes should be incubated at 37° C. When satisfactory growth is obtained, subcultures may be made to Sabouraud's dextrose agar and the cultures grown at room temperature in order to obtain the characteristic slow-growing, glabrous colonies usually described for this species.

*Part II. Studies of the Vitamin Requirements of T. faviforme*

The increased growth and spore production of *T. faviforme* on natural media suggested that these substances are rich in growth factors which may be required by this fungus for its optimal development. The stimulating effect of thiamine added to routine media has been discussed above. Recent studies using synthetic, chemically defined media have shown, in fact, that some strains of *T. faviforme* do require certain of the vitamins, and are stimulated by other growth factors present in natural products.

Schopfer and Blumer (10), in their studies of the growth requirements of an organism which they had isolated and classified as *T. album*, have shown that this strain, although able to grow to some extent on a basal medium without the addition of any vitamin, produced a more rapid growth when biotin was added. This was especially true when a nitrogen source such as ammonium

citrate was used in place of asparagine. They also demonstrated that, in an unbuffered asparagine medium, thiamine, i-inositol, and pyridoxine clearly furthered development.

Robbins, Mackinnon, and Ma (11) studied a Uruguayan strain of *T. discoides* (Mackinnon No. 688) and demonstrated that it required pyridoxine, i-inositol, and molecular thiamine for growth on a basal medium with asparagine as a nitrogen source. Development of this strain was further stimulated by other factors present in complex products such as peptone, casein hydrolysate, hydrolyzed egg albumin, malt extract, gelatine, and a filtrate from white potatoes.

Burkholder and Moyer (12) have shown that a strain which they designated as *T. faviforme* required only two vitamins, thiamine and inositol. They obtained further stimulation by the addition of liver and peptone to their media.

Mackinnon and Artagaveytia-Allende (13) recently compared the vitamin requirements of seven Uruguayan strains. Of these, four required i-inositol, one thiamine, one i-inositol and thiamine, and the seventh (No. 688), previously studied and reported (11), required i-inositol, thiamine, and pyridoxine. It was shown that the vitamin requirements depended on the individual strain, and were not related to any particular colonial form.

#### *A. Growth on basal agar with vitamin additions*

A study was made to determine the vitamin requirements of twenty recently isolated strains of *T. faviforme* (including all three colony forms: varieties album, discoides and ochraceum) as well as of four stock strains: *T. album* (Baudet and Stuhmer), *T. discoides* (Papegaay), *T. ochraceum* (Boedijn), obtained from the Central Bureau for Fungus Cultures, Holland, and *T. discoides* (No. 688), kindly sent from Uruguay by Dr. Juan E. Mackinnon.

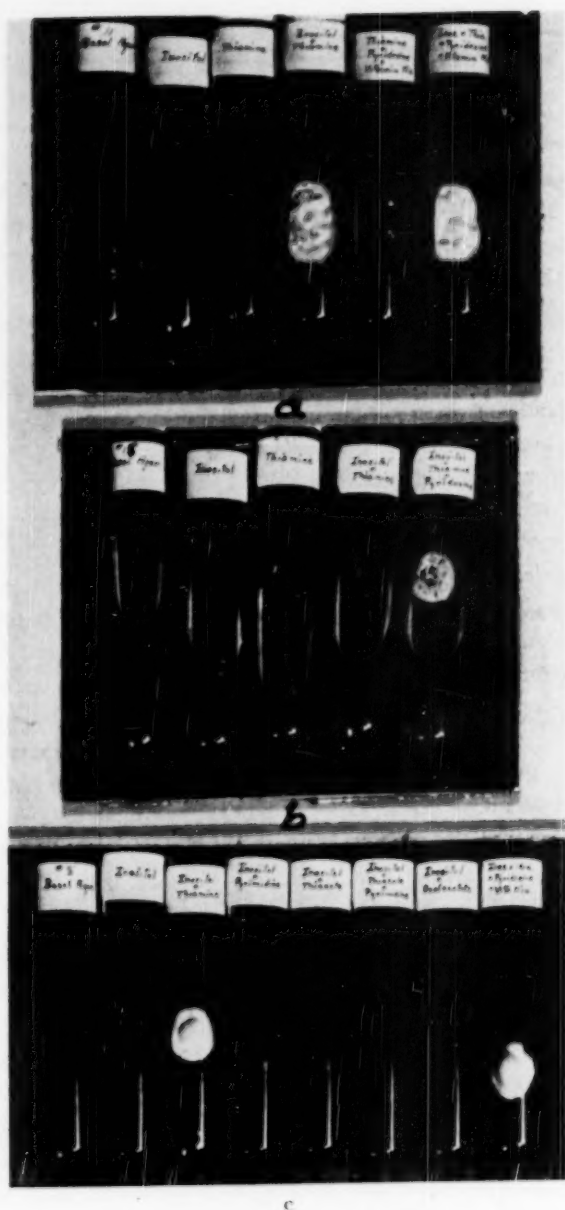
A basal agar was made as follows: 2 g. asparagine (recrystallized 3 times from alcohol), 50 g. dextrose, and 0.1 g.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  dissolved in 1000 ml. Sorenson's phosphate mixture at pH 7 ( $\text{M}/15 \text{KH}_2\text{PO}_4$  and  $\text{M}/15 \text{Na}_2\text{HPO}_4$ ). To this was added 1.5 per cent agar (purified according to the method described by Robbins, 14), and the medium was then autoclaved for 10 min. at

121° C. Seitz-filtered vitamin solutions were prepared at the beginning of each experiment, and equal volumes containing vitamins in varying concentrations and combinations were added to tubes containing 10 ml. of the melted and partially cooled agar. The tubes were then rotated and slanted.

Washed inoculum was obtained from growth in heart infusion broth. It was necessary to wash the mycelium five times before the wash water was free of thiamine. (This was determined by testing the filtrate for ability to support the growth of washed spores of *Phycomyces Blakesleeanus*, which will grow in the presence of minute traces of thiamine.) A small fragment of washed inoculum was placed on the slanted surface of the basal agar with and without the various vitamins tested. All cultures were grown at room temperature for 10 weeks.

The growth studies indicated that: 1. Twenty-one of the strains (twenty recently isolated strains as well as *T. discoides* (Papegaay)) required i-inositol and molecular thiamine; 2. One strain *T. discoides* (No. 688) required pyridoxine in addition to i-inositol and thiamine; and 3. Two strains, *T. album* (Baudet and Stuhmer) and *T. ochraceum* (Boedijn), had no essential vitamin requirements and were not stimulated by additions of vitamins to the basal agar.

For the twenty-one strains which required both inositol and thiamine, inositol when tested alone showed some slight effect. No effect was observed in tubes containing less than 10  $\mu$ g. inositol per ml. but with quantities between 10 and 100  $\mu$ g. per ml. the mycelium developed to a radius of 2 to 10 mm. from the point of inoculation. This was a thick, fern-like subsurface growth. Further increments of inositol did not increase the amount of growth. 100  $\mu$ g. inositol per ml. was therefore considered the maximum effective dosage of this vitamin in an asparagine basal agar. Thiamine showed no effect when tested alone at a concentration of 5  $\mu$ g. per ml. nor even when increased to 500  $\mu$ g. per ml. However, in the presence of 100  $\mu$ g. of inositol per ml., 5  $\mu$ g. of thiamine permitted rapid development of the cultures. The colonies were heaped and frequently folded, with either considerable white surface powder or velvety aerial mycelium. Further increments of thia-

FIG. 5. Strains of *Trichophyton*.

mine did not increase growth. Five  $\mu\text{g.}$  of thiamine per ml. was considered the maximum effective dosage of this vitamin in the asparagine basal agar in the presence of 100  $\mu\text{g.}$  of inositol. None of the other vitamins tested (calcium pantothenate 100  $\mu\text{g.}$  per ml., riboflavin 100  $\mu\text{g.}$  per ml., para-aminobenzoic acid 100  $\mu\text{g.}$  per ml., nicotinamide 100  $\mu\text{g.}$  per ml., biotin 0.05  $\mu\text{g.}$  per ml., choline chloride 100  $\mu\text{g.}$  per ml., and folic acid 100  $\mu\text{g.}$  per ml.) showed any effect when tested singly or in all possible combinations, or when added with the maximum effective doses of inositol and thiamine (FIG. 5a).

The finding that *T. discoides* (No. 688) requires pyridoxine in addition to inositol and thiamine is in accord with the report of Robbins, Mackinnon and Ma (11) who also had studied this strain. Inositol showed some slight effect when tested alone in amounts over 10  $\mu\text{g.}$  per ml., but neither thiamine nor pyridoxine showed any effect except in the presence of the other two essential vitamins. In the presence of the maximum effective doses of inositol and thiamine, 5  $\mu\text{g.}$  of pyridoxine per ml. produced rapid growth of this strain. Five  $\mu\text{g.}$  per ml. was considered the maximum effective dosage of this vitamin in the asparagine basal agar. None of the other vitamins tested showed any stimulating effect (FIG. 5b).

In contrast to all of the other isolates two strains, *T. album* (Baudet and Stuhmer) and *T. ochraceum* (Boedijn), were found to have no essential vitamin requirements and grew well on the basal agar. No stimulation was observed following the addition of inositol, thiamine, pyridoxine, or any of the other vitamins tested.

For all of these strains, basal agar alone, or basal agar with the maximum effective quantities of required vitamins, was not sufficient to produce growth comparable to that obtained on certain natural media. Bacto-neopeptone, beef heart infusion, Bactotryptose, yeast extract and liver extract not only replaced the essential vitamins, where these were required, but in all cases produced a heavier and more rapid growth. This suggests that there are other important nutritional factors present in natural substances which stimulate the growth of this fungus.

Substitution of  $\text{NH}_4\text{Cl}$ , 2 g. per liter, or an acid-hydrolyzed

casein,\* 1 g. per liter, for the asparagine of the basal agar did not change the vitamin requirements of any of the strains, but growth with  $\text{NH}_4\text{Cl}$  was considerably less than with asparagine or casein hydrolysate.

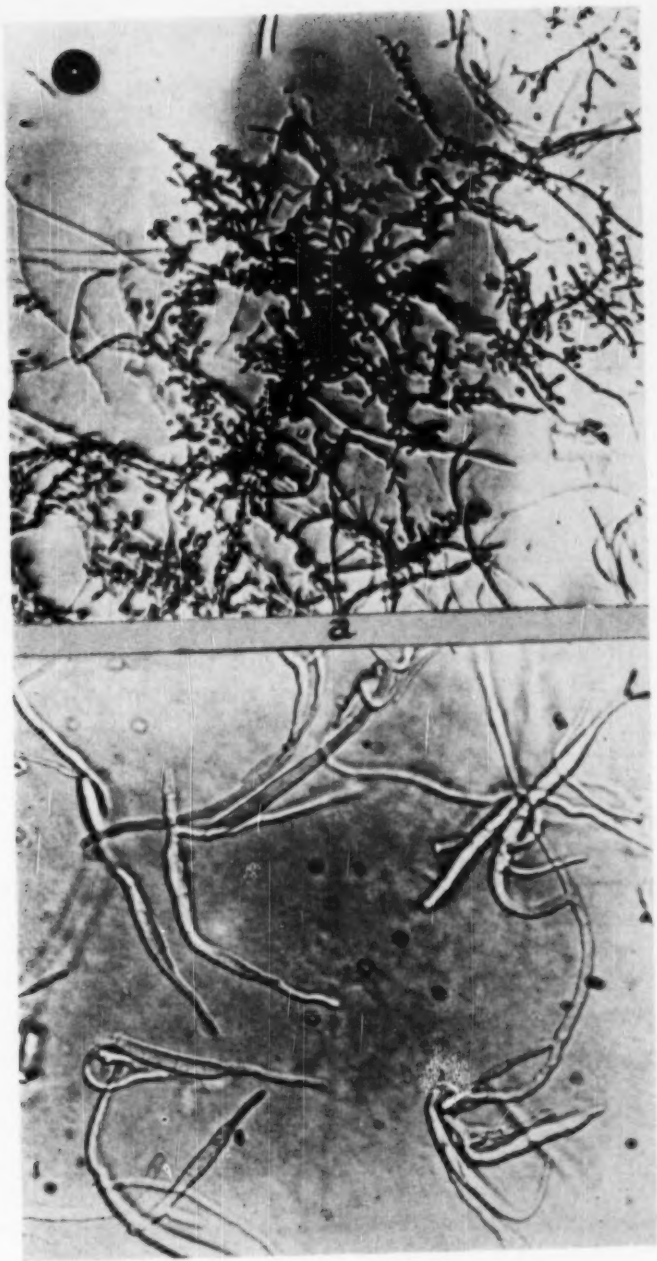
It was found that neither pyrimidine, as 2-methyl-5-ethoxymethyl-6-aminopyrimidine; or thiazole, as 2-amino-thiazole; or an equimolar combination of these two, could substitute for molecular thiamine for any of the strains studied. It was found also that oxalacetate could not be substituted for molecular thiamine (FIG. 5c).

*B. Characteristics of the growth on basal agar with vitamin additions*

The twenty-one strains which required inositol and thiamine produced vigorously growing colonies on the asparagine basal agar to which maximum effective amounts of these two vitamins had been added. The colonies were compact and generally covered with a thick, white, velvety to fluffy surface growth. In some instances a yellow pigment developed in this surface growth as the culture aged. The mycelium appeared to be more regular and better developed than the poorly formed, irregular, twisted mycelium observed in the glabrous colonies on Sabouraud's dextrose agar, and the number of chlamydospores was greatly reduced. All these strains developed microconidia, and in some these were very abundant (FIG. 6a). On the whole, they were more numerous than on the rice grains, on which medium these structures have been described in detail above. Macroconidia were produced, usually in small quantities, by all of these strains on the inositol-thiamine-enriched basal agar. They were characteristically irregular in size and shape, and appeared similar to the macroconidia described on rice grains. One strain produced abundant macroconidia which were long and slender with tapering ends (FIG. 6b).

When 2 g. of  $\text{NH}_4\text{Cl}$  per liter was substituted for asparagine in the vitamin-enriched basal agar, the character of the growth and the number of spores produced were not altered appreciably. However, best growth and spore production were obtained when casein hydrolysate was employed as the nitrogen source.

\* "Vitamin-free" casein, General Biochemicals, Inc., Chagrin Falls, O.



b

FIG. 6. Strains of *Trichophyton*.

*C. Quantitative studies in asparagine basal broth with vitamin additions*

Several strains were employed to determine the amount of growth which could be produced in a liquid synthetic medium with additions of the required vitamins, alone and in combinations; as compared with that obtainable in a natural medium, Difco Heart Infusion Broth. A basal broth was prepared similar to the asparagine basal medium described above except for the absence of agar. Fifty ml. amounts of broth were placed in 150 ml. Erlenmeyer flasks, and freshly prepared vitamin solutions were added in amounts determined from studies on asparagine basal agar: inositol 100  $\mu$ g. per ml., thiamine 5  $\mu$ g. per ml., and pyridoxine 5  $\mu$ g. per ml. Strains No. 1, No. 5, No. 17, No. 18, and No. 19 were chosen as test organisms. Strains No. 1 and No. 5 were recently isolated cultures both of which had shown requirements for inositol and thiamine. Strain No. 17 (*T. discoides* (Papegaay)) also required inositol and thiamine. Strain No. 18 (No. 688, Mackinnon) required inositol, thiamine, and pyridoxine, and strain No. 19 (*T. album*, Baudet and Stuhmer) had no essential vitamin requirements. The flasks were inoculated with tiny shreds of washed mycelium. The cultures were incubated at room temperature and shaken at intervals to maintain a uniform, submerged type of growth. A series of flasks containing 50 ml. of heart infusion broth with and without added vitamins was treated similarly. After ten weeks, the entire contents of the flasks were filtered through sintered glass cups and the retained mycelium washed and then dried for 2 hours at 110° C. The weight of the mycelium was then determined.

Growth of the vitamin-requiring strains corresponded well with that obtained on the basal agar slants in the previous experiment. The strains which had shown growth with inositol and thiamine showed even more clearly here that these vitamins were required, and that the addition of any of the other vitamins tested had no effect on the amount of growth. The weight of mycelium produced with inositol alone, never more than 2 mg., corresponded to the small amount of subsurface growth which had been observed on the basal slants with this vitamin. For all strains, approxi-

mately twice as much growth was produced in the heart infusion broth as in the basal broth with added vitamins. However, even more growth was obtained when the same doses of the required vitamins were added to the heart infusion broth. This indicated that the heart infusion broth did not contain the required vitamins in sufficient amounts to produce maximum growth in this medium. Table 1 gives a summary of the results of these tests.

Titration were made in the asparagine basal broth to determine the minimum concentrations of the essential vitamins that would stimulate the growth of these several strains of *T. faviforme*.

Asparagine basal broth, prepared as described above, was placed in 10 ml. amounts in test tubes (150 × 19 mm.) and sterilized by autoclaving. Ten ml. of the vitamin to be tested was added aseptically to one of these tubes and diluted serially through 30 tubes, the amount of vitamin being halved by each transfer. The following titrations were carried out for each strain: 1. Titration with inositol alone; 2. Titration of inositol in the presence of thiamine (50 µg. thiamine being added to each tube at the completion of the dilution process); 3. Titration of thiamine in the presence of inositol (1000 µg. of inositol being added to each tube at the completion of the dilution process); and 4. Titration of pyridoxine in the presence of both inositol and thiamine. Control tubes contained asparagine broth, or the basal broth with each vitamin singly and in all combinations in maximum effective amounts. All tubes were brought to equal volume with sterile distilled water. Inoculations were made with fragments of washed mycelium and the cultures were allowed to grow at room temperature for 6 weeks.

Titration 1 indicated that for strains No. 1, No. 5, and No. 18, inositol showed some slight effect alone. The amount of growth was very small and appeared to be the same in all tubes showing growth. The minimal effective concentration ranged from 12.2 to 97.5 µg. per 10 ml. of asparagine broth, depending on the strain. Titration 2 showed, for strains No. 1 and No. 5, that in the presence of adequate quantities of thiamine the amount of growth was greatly increased, and tubes bearing the same dilutions of inositol which alone had produced only a small submerged fluffy ball of mycelium, were now filled with growth. Growth fell off sharply

TABLE 1  
COMPARISON OF GROWTH OF *T. fariniforme* ON BASAL ASPARAGINE BROTH AND HEART  
INFUSION BROTH WITH AND WITHOUT VITAMINS

Strain	Thiamine 5 µg./ml.	Pyridoxine 5 µg./ml.	Inositol 100 µg./ml.	Inositol and thiamine	Inositol, thiamine, and pyridoxine	Thiamine- pyridoxine + "vitamin mixture"	Inositol- thiamine- pyridoxine + "vitamin mixture"	Heart infusion broth + inositol thiamine pyridoxine	Heart infusion broth (control)
No. 1 (album variety) Penna.	0	0	less than 1 mg.	14.61 mg.**	14.39 mg.**	0	15.96 mg.**	39.06 mg.**	0
No. 5 (discoides variety) Penna.	0	0	1.59 mg.**	17.51 mg.**	18.34 mg.**	0	18.16 mg.**	91.70 mg.**	0
No. 17 T. discoides (Papageau) Holland	0	0	1.76 mg.**	29.17 mg.**	28.96 mg.**	0	27.60 mg.**	85.15 mg.**	0
No. 18 T. discoides (Mackinnon) Uruguay	0	0	1.4 mg.**	1.35 mg.**	19.70 mg.**	0	17.17 mg.**	56.85 mg.**	0
No. 19 T. album (Baudet-Stuhmer) Holland	29.18 mg.*	27.62 mg.*	30.0 mg.*	31.19 mg.*	30.45 mg.*	27.15 mg.*	25.20 mg.**	73.83 mg.*	28.23 mg.**

\* Average of 4 weighings—dried mycelium from 4 flasks.

\*\* Average of 6 weighings—dried mycelium from 6 flasks.

"Vitamin mixture": (µg. per ml.), pantothenate 100, riboflavin 100, biotin 0.05, nicotinamide 100, para-aminobenzoic acid 100, choline 100, and folic acid 100.

at the same dilutions of inositol as in the first titration. Titration 3 showed, for strains No. 1 and No. 5, that in the presence of adequate amounts of inositol the amount of growth is proportional to the amount of thiamine present with maximum growth occurring in tubes containing 0.2  $\mu$ g. thiamine. It was noted that large amounts of thiamine—up to 5.0 mg.—had no inhibitory effect. The lower end point of the effect of thiamine occurred in concentrations of 0.02 and 0.04  $\mu$ g. thiamine per 10 ml. asparagine broth. Beyond these there occurred only a small amount of growth, which could be accounted for by the inositol present. Titration 4 indicated, for strain No. 18, that in the presence of adequate amounts

TABLE 2  
SMALLEST AMOUNTS OF THE ESSENTIAL VITAMINS WHICH WILL  
STIMULATE THE GROWTH OF SEVERAL STRAINS OF *T. faviforme*  
IN 10 ML. OF ASPARAGINE BASAL BROTH

Strain	Inositol	Thiamine (in the presence of 1000 $\mu$ g. inositol)	Pyridoxine (in the presence of 1000 $\mu$ g. inositol, and 50 $\mu$ g. thiamine)
	$\mu$ g.	$\mu$ g.	$\mu$ g.
No. 1 (album variety) Penna.	97.5	0.02	No effect
No. 5 (discoides variety) Penna.	12.2	0.04	No effect
No. 18 <i>T. discoides</i> Mackinnon Uru- guay	48.0	No effect	0.02
No. 19 <i>T. album</i> (Baudet-Stuhmer) Holland	No effect	No effect	No effect

of both inositol and thiamine, pyridoxine was effective in concentrations as low as 0.02  $\mu$ g. per 10 ml. asparagine broth. Strain No. 19, which had shown no vitamin requirements in previous studies, grew equally well in control tubes containing basal broth and in tubes containing the vitamins (Table 2).

#### SUMMARY

1. As a result of cultural studies employing single spore strains of recently isolated, large-spored, ectothrix trichophytons of animal

origin, it is apparent that *Trichophyton album*, *T. discoides*, and *T. ochraceum* are variants of a single species. It is proposed that they be classified as *T. faviforme* (var. *album*, *discoides*, and *ochraceum*).

2. Growth of these strains is very slow and poor on Sabouraud's dextrose agar, and the small, glabrous colonies produced are composed of masses of poorly formed mycelium and numerous chlamydospores. Special enriched media are described which allow rapid development of these forms with the production of heavy, folded colonies with considerable powdery or downy white surface growth. On such media the mycelium is more regular in form and bears fewer chlamydospores. The aerial growth produces large numbers of microconidia, and, in most strains, macroconidia were also found. It would seem that the morphological characteristics usually described for this species on Sabouraud's dextrose agar are the results of inadequate nutrition.

3. Vitamin-free, asparagine basal media were used in studies of the vitamin requirements of freshly isolated strains as well as of stock strains of *T. faviforme*. Twenty-one recently isolated strains were shown to require i-inositol and molecular thiamine. One stock strain, *T. discoides* (Papegaay), had similar requirements, while two others, *T. album* (Baudet and Stuhmer) and *T. ochraceum* (Boedijn), were autotrophic for the vitamins. *T. discoides* (No. 688 Mackinnon), reported to require i-inositol, molecular thiamine, and pyridoxine by Robbins, Mackinnon, and Ma (11), was compared with these strains.

4. Rapid growth with characteristic spores was obtained on synthetic media to which had been added adequate amounts of the required vitamins. The amount of growth, however, was much less than that produced on a natural medium. The minimal effective quantities of the vitamins were determined by serial dilution methods.

5. Recent reports (6, 15, 16) indicate that *T. faviforme* is not an uncommon agent of ringworm in this country. It is prevalent particularly in cattle raising areas, and human infections can usually be traced to contacts with infected cattle.

6. In view of the fact that *T. faviforme* has requirements for

optimal growth which are not supplied by the usual cultural procedures and media, it is recommended that special vitamin-enriched media with added antibiotics, and incubation at 37° C., be used for attempted isolation of this fungus.

The author wishes to express her gratitude to Dr. Rhoda W. Benham of the Department of Dermatology, Columbia University, College of Physicians and Surgeons, for advice and assistance in this work.

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## ILLUSTRATIONS

FIG. 5. *a*, *T. faviforme* (strain No. 11) which requires inositol and thiamine. Addition of pyridoxine or mixture of other vitamins does not change amount of growth; *b*, *T. discoides* (No. 688 Mackinnon) which requires pyridoxine in addition to thiamine and inositol; *c*, *T. faviforme* (strain No. 8) requires inositol and thiamine. The molecular thiamine cannot be replaced by thiazole, pyrimidine, a combination of these or oxalacetate.

FIG. 6. *a*, *T. faviforme* (strain No. 5) on basal agar with inositol and thiamine. Note large numbers of microconidia; *b*, *T. faviforme* (strain No. 8) on basal agar with inositol and thiamine. Note large numbers of macroconidia.

## THE NUTRITIONAL REQUIREMENTS OF *EREMOTHECIUM ASHBYII* GUILL.\*

EUGENE L. DULANEY AND F. H. GRUTTER

In his descriptions of *Eremothecium ashbyii*, Guilliermond (1, 2) noted the production of large quantities of a pigment that colored the mycelium yellow and diffused into the culture medium. Within the cells, crystals of the yellow pigment were found in the vacuoles. In another publication, Guilliermond, Fontaine and Raffy (3) classified this pigment as a flavin and commented on its similarity to riboflavin. This flavin was later extracted and crystallized by Raffy and Mirimanoff (4, 5).

Although *E. ashbyii* has attained some industrial importance as a source of riboflavin, there have been few publications concerning the physiology of this species. One such paper is that of Schopfer (6) and it is this publication that we would like to refer to at this time.

Schopfer found that *E. ashbyii* would grow in natural media but not in a synthetic medium containing glucose 1%, glycine or asparagine 0.1%, magnesium sulfate 0.05% and acid potassium phosphate 0.15%. When various peptones were added singly to this medium, growth and riboflavin production occurred. The response varied with the peptone, and treatment of peptone with norite yielded a filtrate that did not stimulate growth when added to the synthetic medium noted above. When the norite-treated peptone plus biotin was added to the medium containing glucose and glycine, growth and riboflavin production occurred. In addition, vitamin B<sub>1</sub> and *D*-inositol were found to be complementary factors in that they intensified the effect of the biotin in this medium; however, they were not essential as was the biotin. It should be emphasized that no growth occurred in the synthetic medium supplemented only with the three vitamins or with the norite-treated

\* Contribution from the Research Laboratories of Merck & Co., Inc., Rahway, N. J.

peptone. In order to obtain growth, it was necessary to add both the norite-treated peptone and the vitamins, at least biotin, to the medium.

Recently we have been investigating the nutritional requirements of *E. ashbyii* and in particular its vitamin requirements. Although the experimental conditions used by us are not strictly the same as those used by Schopfer (6), it appears desirable to present some of our results at this time.

#### MATERIALS AND METHODS

We have used a basal medium containing glucose 1.0%, acid hydrolyzed casein 1.0%,  $K_2HPO_4$  0.2%,  $MgSO_4 \cdot 7H_2O$  0.1%, NaCl 0.02%,  $CaCl_2$  0.02%,  $FeSO_4 \cdot 7H_2O$  0.0005%,  $ZnSO_4 \cdot 7H_2O$  0.0005% and distilled water to volume. The casein used was the commercial product Labco sold by the Borden Co. as being vitamin-free. Before use it was acid hydrolyzed, neutralized with barium hydroxide and treated twice with norite. This basal medium was dispensed in 25 ml. amounts in 125 ml. Erlenmeyer flasks. All glassware used in these experiments was acid cleaned and thoroughly rinsed in distilled water. To this basal medium, biotin (0.04  $\mu g./ml.$ ), vitamin B<sub>1</sub> (0.1  $\mu g./ml.$ ) and *i*-inositol (0.2 mg./ml.) were added singly and in all possible combinations. The flasks were capped with heavy aluminum foil and sterilized by autoclaving at 15 lbs. pressure for 17 minutes. Sterile cotton plugs were used to replace the aluminum foil caps after autoclaving.

Two cultures of *E. ashbyii* were used in some of these experiments. One of these was obtained directly from Guilliermond and the other came from the Centraalbureau voor Schimmelcultures at Baarn. Schopfer notes in his 1944 publication that the culture used in his experiments came from Baarn. The 1943 Baarn catalogue lists only one culture of *E. ashbyii* and this is the culture included in some of our experiments. Both of these cultures have been in the Merck culture collection for several years. It should be emphasized, however, that there is no certainty that we have used the same strain of *E. ashbyii* that Schopfer used.

The inoculum for these experiments was grown in 25 ml.

amounts of yeast-extract glucose broth in 125 ml. Erlenmeyer flasks. After 48 hours growth on a rotary shaker, the mycelium was centrifuged and washed four times with sterile distilled water, then made up to one half the original volume with sterile distilled water. Three drops of this suspension were used to inoculate each flask. The incubation temperature for all experiments was 28° C. All flasks were incubated on rotary shakers moving at 220 rpm and describing a horizontal circle one and one half inches in diameter. In addition, replicate flasks of the treatments concerning the effects of vitamins were incubated statically.

The riboflavin content of the metabolism solutions was determined fluorometrically.

Three replicate flasks were used for each treatment and the experiments have been repeated several times.

#### RESULTS AND DISCUSSION

Comparable results have been obtained in experiments incubated statically and on rotary shakers. The results of a representative experiment concerning the effects of adding biotin, *i*-inositol and vitamin B<sub>1</sub> to the basal medium, singly and in combination, are shown in TABLE I. The culture of *E. ashbyii* used in this experiment was the one obtained from Guilliermond. The flasks were incubated on a shaker.

TABLE I  
THE EFFECT ON RIBOFLAVIN PRODUCTION OF ADDING BIOTIN,  
VITAMIN B<sub>1</sub>, AND *i*-INOSITOL, SINGLY AND IN COMBINATION,  
TO THE FERMENTATION MEDIUM

Treatment	Riboflavin broth potency μg./ml. after			Dry wt. mg./ml. after 7 days
	3	5	7 days	
Basal medium	5	6	8	
B.M. plus biotin	5	6	6	
B.M. plus vitamin B <sub>1</sub>	9	8	11	
B.M. plus <i>i</i> -inositol	40	57	97	5.3
B.M. plus biotin and vitamin B <sub>1</sub>	7	8	10	
B.M. plus biotin and <i>i</i> -inositol	61	67	86	4.2
B.M. plus vitamin B <sub>1</sub> and <i>i</i> -inositol	46	79	105	3.2
B.M. plus biotin, vitamin B <sub>1</sub> and <i>i</i> -inositol	73	69	82	4.3

Growth in the basal medium was very sparse when measured visually. There may have been some slight cell proliferation but it was so small that dry weight measurements were not made. In addition, biotin and vitamin B<sub>1</sub>, when added singly or in combination to the basal medium, did not increase the amount of growth or riboflavin production over that obtained in the basal medium. In these treatments, growth, if it occurred, was so slight that it was not measured. There was, however, some riboflavin production in these treatments. A level of approximately 7 µg./ml.-10 µg./ml. was reached by the third day after which there was little increase. It may be noted that the dry weight of cells added to each flask as inoculum was approximately 1.5 mg. Apparently some stimulatory substances and possible intermediates in the biosynthesis of riboflavin were carried over with the inoculum in amounts sufficient to allow some cell proliferation as well as chemical transformation to riboflavin. When the small amount of mycelium in the flasks showing slight growth and low riboflavin levels was removed aseptically by centrifugation, washed with sterile distilled water and used to inoculate other flasks containing media with the same vitamin supplements, no observable growth or riboflavin production occurred.

Growth and riboflavin production, however, were obtained in the basal medium supplemented with *i*-inositol alone or in the media supplemented with any combination of vitamins that included *i*-inositol. The addition of biotin and vitamin B<sub>1</sub> to the basal medium containing *i*-inositol did not result in an increase in the maximum riboflavin level over that obtained in the basal medium containing *i*-inositol alone. There was, however, an apparent earlier increase in riboflavin level than occurred in the basal medium supplemented only with *i*-inositol.

In all of the treatments in which growth occurred the experiment was carried through three passages. Comparable results were obtained with the Baarn culture of *E. ashbyii*.

These results indicate that *E. ashbyii* has an absolute requirement for *i*-inositol. They do not, however, indicate an absolute requirement for biotin or vitamin B<sub>1</sub>. There would seem to be a possibility that the hydrolyzed casein contained low levels of biotin

even after the treatments given it. However, no biotin could be demonstrated by microbiological assay using an organism having a requirement for biotin. By the same method, vitamin B<sub>1</sub> could not be detected in the hydrolyzed casein. In addition, when L (-) proline, DL glutamic acid and L (+) arginine were substituted singly for the hydrolyzed casein in the basal medium, good growth and riboflavin production occurred if only *i*-inositol was used as a supplement. It is possible that the natural amino acids such as L (-) proline and L (+) arginine could be contaminated with biotin. The DL glutamic acid, however, was produced by organic synthesis and there is very little likelihood of its being contaminated with vitamins.

An observation may be made about the failure of Schopfer to obtain growth in his synthetic medium to which biotin, vitamin B<sub>1</sub> and *i*-inositol were added. This medium contained only glycine as a source of nitrogen. The two cultures of *E. ashbyii* used in our experiments will not use glycine as a sole source of nitrogen. It may be that the norite-treated peptone that had to be added to this medium containing these three vitamins in order to obtain growth was merely supplying an available source of nitrogen. When we substituted L (-) proline, L (+) arginine or DL glutamic acid for the glycine in Schopfer's synthetic medium, though at a higher level than 0.1%, growth and riboflavin production readily occurred if only *i*-inositol was added. No other absolute growth requirement could be demonstrated.

In all of the above experiments, the level of riboflavin produced is much lower than that obtained in more complex organic production media. These low levels of riboflavin are due, in part, to the low concentrations of carbon and nitrogen employed in these defined media. It may well be, however, that as yet unidentified factors exist that stimulate production of riboflavin by *E. ashbyii*. The following compounds, however, when added singly to the basal medium plus *i*-inositol did not induce any increase in riboflavin production: glutamine 5.0 µg./ml., pantothenic acid 1.0 µg./ml., vitamin B<sub>12</sub> 0.001 µg./ml., nicotinic acid 10.0 µg./ml., pyridoxine 10.0 µg./ml., choline 5.0 µg./ml., para aminobenzoic acid 1.0 µg./ml. and folic acid 0.1 µg./ml.

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## A NEW SPECIES OF GELASINOSPORA<sup>1, 2</sup>

CONST. J. ALEXOPOULOS AND SUN HUANG SUNG

(WITH 6 FIGURES)

### INTRODUCTION

The genus *Gelasinospora*, closely related to *Sordaria* and *Neurospora*, was erected by Dowding (1) in 1933 to include two species of Pyrenomycetes with dark, pitted ascospores, *Gelasinospora cerealis*, and *Gelasinospora tetrasperma*, described by her at that time. A third species, *Gelasinospora autosteira*, found growing on Spanish moss in Natchez, Mississippi, in March 1949, is now added.

*Gelasinospora autosteira* differs from *Gelasinospora tetrasperma* in that its asci are normally octosporous, from *Gelasinospora cerealis* in its somewhat larger perithecia and in the smaller size of its ascospores, and is unlike both species in that it is composed of two strains, self-sterile and inter-fertile, designated A and B.

### SPECIES DIAGNOSIS

#### *Gelasinospora autosteira* spec. nov.

Mycelium ramosius, hyphae 3-14.6  $\mu$  diam.; perithecia superficialia, subglobosa, membranacea, 0.42-0.61 mm. diam., 0.65-0.71 mm. alta, atra, glabra, rostro cylindrico et prominente; asci octospori, aparaphysati, cylindrici, 14.1-18.3  $\mu \times$  218.6-244  $\mu$ ; ascospori primo hyalini, deinde fusco-brunnei vel atri, opaci, faveolati, 10.6-14.1  $\mu \times$  16.7-26.5  $\mu$ , binucleati; conidia et spermatia incognita.

Thalli auto-incompatibiles; species consistens ex duabus lineis designatis A et B.

Melrose Estate, Natchez, Mississippi, U. S. A., 20 Martii, 1949.

Hab. in caulibus *Tillandsiae usneoidous*.

Mycelium profusely branched, hyphae 3-14.6  $\mu$  diam.; perithecia superficial, pyriform, membranous, 0.42-0.61 mm. diam., 0.65-

<sup>1</sup> Contribution No. 50-3 from the Department of Botany and Plant Pathology, Michigan State College.

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0.71 mm. tall, black, glabrous; beak prominent, cylindric, characteristically bent or hooked at the tip; asci normally octosporous, cylindric, persistent,  $14.1\text{--}18.3\ \mu \times 218.6\text{--}244\ \mu$ ; ascus walls thickened at the apex around a central canal which terminates in a round pore; paraphyses lacking; ascospores oval, hyaline and transparent when young, changing to yellow and finally to very dark brown or black, opaque,  $10.6\text{--}14.1\ \mu \times 16.7\text{--}26.5\ \mu$ , binucleate at maturity; characteristic pits on ascospore walls best visible just before maturity of ascospores has been attained; conidia and spermatia unknown.

Thalli self-incompatible, species consisting of two inter-fertile strains designated A and B.

Collected at Melrose Estate, Natchez, Mississippi, U. S. A., on March 20, 1949, and developed to maturity in moist chamber.

On stems of *Tillandsia usneoides*. Specific epithet *autosteira* (*auto* = self + *steira* = fem., sterile) refers to the self sterility of thalli derived from the growth of single ascospores.

Portions of the type specimen deposited in the herbarium of Michigan State College, the New York Botanical Garden, and the British Museum.

Cultures of A and B strains deposited with the American Type Culture Collection, Washington, D. C., the Centraalbureau voor Schimmelcultures, Baarn, Holland, and the Commonwealth Mycological Institute, Kew, Surrey, England.

#### MATERIALS AND METHODS

The fungus was first isolated in early April 1949 from a specimen of *Tillandsia usneoides* collected three weeks earlier at Natchez, Mississippi, while the senior author was visiting the southern United States. The *Tillandsia* specimen was collected and placed in a large phial in the hope that Myxomycete fructifications might develop thereon in a moist chamber. Instead, perithecia of a Pyrenomycete (FIG. 5), clearly belonging to the genus *Gelasinospora*, were observed when the specimen was examined upon return to East Lansing.

The observations reported below are based on cultures derived from single ascospore isolations. The isolations were made with the aid of fine, glass needles which were used to isolate ascospores

under the dissecting microscope at 36 or 72 magnifications. For serial isolations from a single ascus, the ascus was isolated on a sterile slide, transferred to corn meal agar in a Petri dish, and the ascospores pushed out in series. They were then transferred to separate Petri dishes and allowed to form colonies from which transfers were made to agar slants for stock cultures.

Three series of isolations were made. Series G-1, 2, 3, and 4 consisted of four monosporous isolates obtained from random isolations of ascospores which had been ejected from the perithecia in the moist chamber and had fallen on the filter paper on which the material was resting. From a number of ascospores placed on corn meal agar, only four germinated yielding the above series. Series GOa-1, 2, 3, 5, 6, 7, and 8 was obtained when eight ascospores were isolated in series from a single ascus on corn meal agar and heated at 60° C. for thirty minutes. All but No. 4 germinated and yielded stock cultures. Series Gt-1, 2, 3 and 4 was obtained from an ascus that contained only four ascospores near the upper portion. Although ordinarily tetrasporous asci also contain the remnants of the four aborted spores, in this one no aborted spores were evident. It is possible that four ascospores had escaped leaving the other four still in the ascus. All four of these ascospores germinated after thirty minutes in the drying oven at 60° C.

Difco corn meal agar alone or with 2% Difco malt extract added was used for the experiments reported below.

For comparison with *Gelasinospora cerealis*, the other octosporous species in this genus, a culture (Winnipeg isolation) obtained through the kindness of Dr. E. Silver Keeping was employed.

For staining ascospore nuclei, the propiono-carminic smear method, as described to the senior author by Dr. L. S. Olive and modified by the junior author, was used. The method consists, briefly, in fixing a block of agar bearing perithecia in a mixture of 3 parts absolute alcohol and 1 part propionic acid over night, transferring to 4% iron alum for ten minutes, washing with running water for about 4 hours, and crushing the perithecia on clean slides in propiono-carminic prepared in accordance with the directions given by Sass (5) for aceto-carminic, but substituting propionic acid for acetic acid. After the cover slip was in place, the

mount was heated over a flame several times, sealed with glycerine jelly, and stored at room temperature for a few days, until the nuclei were well stained.

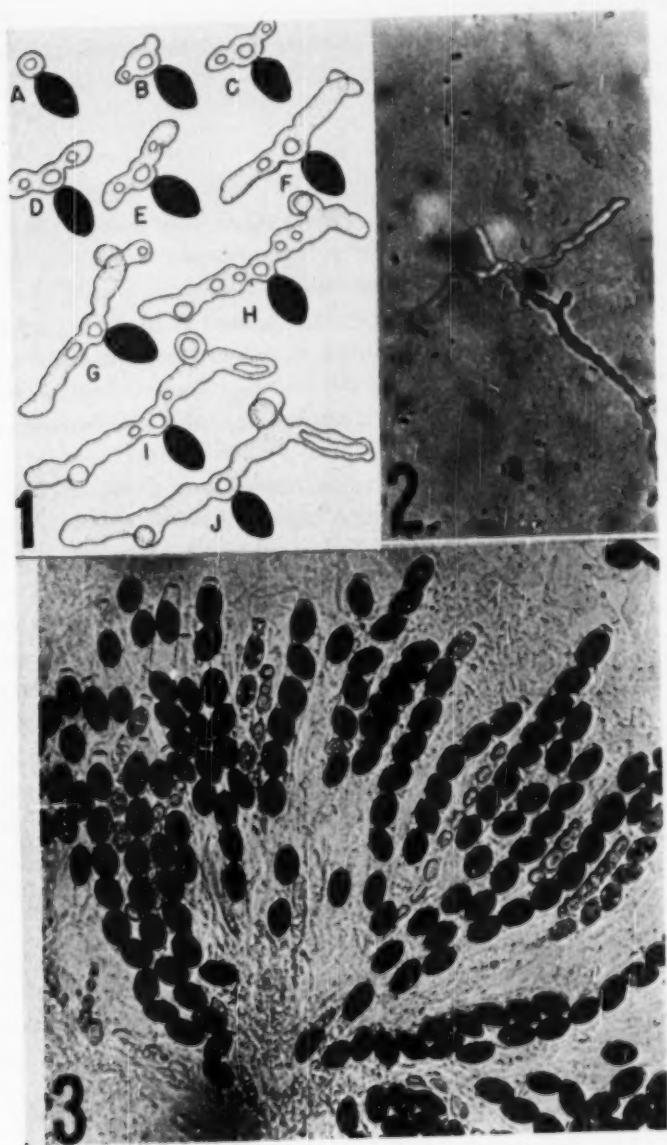
#### ASCOSPORE GERMINATION

The ability of the ascospores of *Gelasinospora autosteira* to germinate is apparently an inherited characteristic as it has been shown to be for *Neurospora* by Lindegren (4). Some ascospores will germinate much more readily than others and some will respond to heat treatment better than others. It appears also that the ascospores resulting from certain crosses germinate more easily than those from other crosses. These phases of the problem, however, have not been systematically investigated.

Dowding (1) reports 26% germination of unheated spores of *Gelasinospora cerealis* and *Gelasinospora tetrasperma*. Only 9% germination was obtained with *Gelasinospora autosteira* when 100 untreated spores, selected at random, were placed on corn meal agar. Treatment with biotin, freezing, placing at 58° C. for 30 minutes, and the combination of freezing and heating, failed to increase the percentage of germination in this experiment. In view of the above, it was particularly fortunate that seven of the eight spores in series GOa and all four of the spores in series Gta germinated.

In subsequent experiments, the details of which will be reported in a later paper, it was discovered that the method successfully employed by Dodge (3) for various Ascobolaceae considerably increased the percentage of spore germination in *Gelasinospora autosteira*. This method, as applied here, consists of placing spores, resting on the surface of corn meal agar, in a drying oven and slowly raising the temperature so that it reaches 70° C. in about 30 minutes. The heat is then turned off and the spores are permitted to remain in the oven until the temperature falls to 35° C. or below.

Ascospore germination on corn meal agar at room temperature begins with the formation of a vesicle four and one-half to five hours after planting. Growth is generally rapid after this and the entire surface of the agar in a Petri dish is often covered in about



FIGS. 1-3. A new species of *Gelasinospora*.

three days from the growth of a single ascospore. Figure 1, A-J, shows various stages in the germination of an ascospore. Such germination is very similar to that described by Dowding (1) for *Gelasinospora tetrasperma*.

#### MYCELIAL CHARACTERS

The mycelium of *Gelasinospora autosteira*, as it develops on corn meal agar, consists of rapidly growing hyphae of various thicknesses. The majority of the hyphae in a mature colony are about  $4\ \mu$  in diameter, and irregularly septate, their cells varying considerably in length. Larger hyphae of an average diameter of  $8\ \mu$  constitute the main strands of the colony. The septa in these are placed more regularly, the average length of the cells being somewhere between 25 and  $35\ \mu$ . Giant hyphae are occasionally seen measuring  $14.5\ \mu$  in diameter. The hyphal cells are probably coenocytic as they have been shown to be in *Gelasinospora tetrasperma* by Dowding and Buller (2). The mycelium was stained in 0.05% Heidenhain's haematoxylin after being fixed in Duggar's Gilson's fluid to which a few urea crystals had been added, in accordance with Dowding and Buller's recommendation. A large number of what appeared to be nuclei were stained in each cell, but these structures were so small that it was impossible to identify them with certainty.

The fungal colony is colorless at first, but in most monosporous isolates under study an over all brown color begins to manifest itself when the colony is about a week old. The intensity of the color varies with the isolate and may be linked with the compatibility factor according to present indications. On agar containing malt extract, the brown color becomes very dark.

Aerial mycelium is produced abundantly in media containing malt extract. It covers the colony completely and grows up the sides of the Petri dish and in columns from the surface of the agar, sealing the cover to the bottom of the dish. Abundant aerial mycelium often exhibits a pink color.

On corn meal agar, some aerial mycelium is also produced, generally in small tufts over the surface of the agar. Corn meal agar is preferable for some types of study and is now being used by the

writers almost exclusively for growing this fungus because of its tendency to suppress formation of aerial mycelium, thereby making archicarps and perithecia more easily visible.

#### PERITHECIAL PRODUCTION

The ascocarps of *Gelasinospora autostreia* are pyriform, black perithecia with a prominent, hooked beak. On the natural host the perithecia are glabrous, but in culture the perithecial body is covered with many gray hairs, the beak alone being glabrous. In size, the perithecia are somewhat greater in diameter than those of *Gelasinospora cerealis*, measuring 0.42–0.61 mm.

Isolates G-1, G-2, G-3, and G-4, resulting from single ascospores selected at random, were grown singly and in all possible combinations in Petri dishes, on corn meal agar with 2% malt extract. None of the single isolates yielded perithecia, nor did the G-1  $\times$  G-2, and the G-3  $\times$  G-4 crosses. However, when G-1 and G-3, or G-2 and G-4 isolates were grown together, clusters of perithecia developed near the edges of the Petri dishes on the line of contact between the two mycelia. This experiment was repeated several times with the same results. It was concluded, therefore, that this fungus consists of two self-incompatible strains and that isolates G-1 and G-2 were of one strain while G-3 and G-4 were of the other. This is in contrast to the condition found by Dowding (1) in the other two *Gelasinospora* species which are both homothallic except for the self-incompatible strains derived from dwarf spores of *Gelasinospora tetrasperma*.

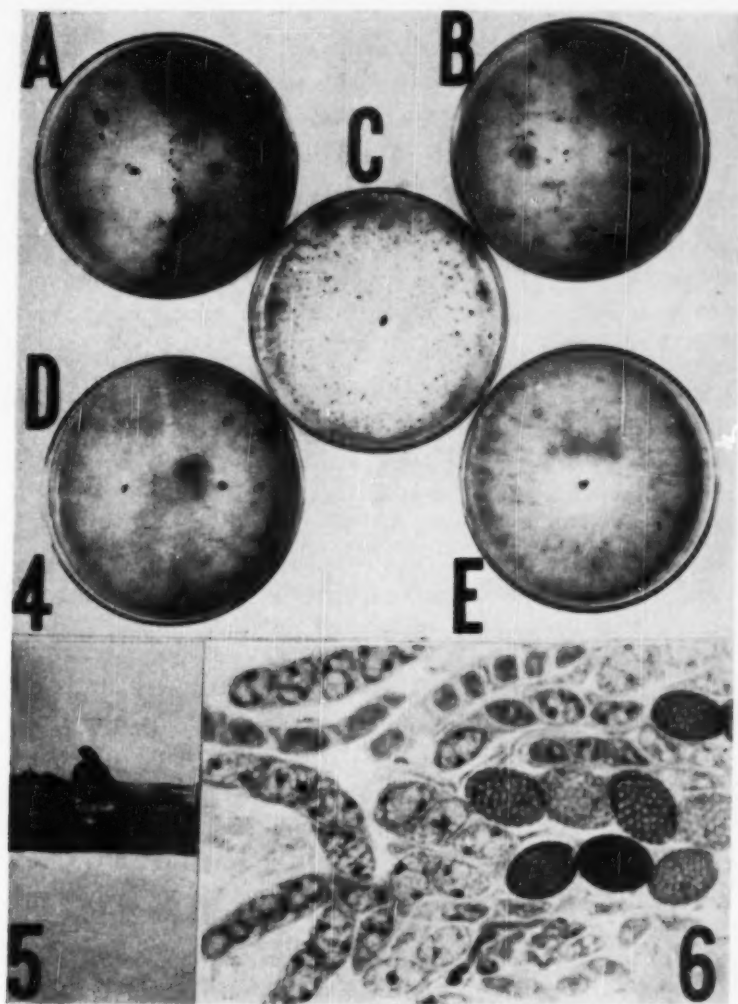
Isolates GOa-1, GOa-2, GOa-3, GOa-5, GOa-6, GOa-7, and GOa-8, all obtained from a single ascus, were similarly tested as single colonies and in all possible combinations. None of the single isolates yielded perithecia, nor did any combination between the first three or the last four isolates in the series. Some single colonies produced many minute archicarps, just visible to the naked eye, which remained undeveloped. All combinations between any one of the first three and any one of the last four yielded numerous perithecia, generally confined to the edge of the Petri dish in the region where the two mycelia met. This experience, repeated several times with the same results, constitutes further evidence that

*Gelasinospora autosteira* is not homothallic. It is more than probable that had GOa-4 germinated it would have been found to belong to the same strain as GOa-1, 2, and 3, the compatibility factor having segregated in this ascus in the first division.

A further test was made with Gta-1, 2, 3, and 4, isolated from an ascus containing only four ascospores. These were grown singly and in all possible combinations with no perithecia resulting in any case. If the assumption made earlier is correct, that the first four ascospores of this ascus had already escaped at the time of isolation, the result of these crosses would indicate that the compatibility factor segregated in the first nuclear division in this ascus as well as in the one which yielded the GOa series.

In *Gelasinospora autosteira*, as in *Gelasinospora tetrasperma* (2), nuclei from the A and B strains probably come together by means of somatogamy, no spermatia or conidia having been found in any of the three known species in this genus. When two compatible mycelia are mated in a Petri dish, the perithecia which are formed are generally located at some point or points of contact between the two colonies, or, in some cases, all along the line of contact (FIG. 4A). Perithecial development is often confined to one or two small areas at the margin of the dish on the line of contact between the two mycelia. This may be a response to the greater oxygen supply at the margin of the dish. In some cases no more perithecia develop, but in others, a second crop matures nearer the center of the dish following more or less the line of contact of the two mycelia (FIG. 4B). Dowding and Buller (2) state that *Gelasinospora tetrasperma* will not form perithecia in sealed Petri dishes. They found it necessary to grow the fungus in open Petri dishes in order to induce perithecial formation. Although *Gelasinospora autosteira* appears to prefer an abundance of oxygen for fruiting, as evidenced by the location of perithecia near the margin of the Petri dish when aerial mycelium is very abundant, the fungus is apparently not as sensitive to the amount of oxygen present as is *Gelasinospora tetrasperma* since it never fails to produce perithecia in closed Petri dishes, other conditions being favorable.

At room temperature, near a window, but without the aid of artificial light at any time, perithecia become clearly visible in about



FIGS. 4-6. A new species of *Gelasinospora*.

14 days after planting. Archicarps, when formed, usually appear in four to five days after planting and are barely visible to the unaided eye. The time of perithecial development, however, varies considerably and may be correlated with certain parental crosses and environmental conditions, as may also be the number of peri-

thecia formed, and their distribution in a Petri dish. These phases of the problem are under investigation.

Ascospores begin to mature about one month after planting. Forcible ejection of ascospores begins about a week after the first ascospores have matured as evidenced by their dark color.

Isolates which have been kept in culture by mycelial transfers for a few months lose their ability to produce perithecia altogether, and fertile strains may be reestablished only by germinating more ascospores. It is therefore advisable to have mixed cultures with perithecia always on hand if one is to do any considerable work with this fungus.

#### ASCI AND ASCOSPORES

Asci are produced abundantly in the perithecia of *Gelasinospora autosteiira*. They are long and cylindrical. When pressed out of the perithecium under a cover slip, they spread out from a common center in a typical fashion (FIG. 3). There are no paraphyses. The asci are normally octosporous, but spore abortion is common. Asci with 3, 4, 5, 6, or 7 mature spores, the remaining aborted, can be found in almost any perithecium, but usually when abortion occurs, four spores abort and four develop.

Although there is some variation in the size of the ascospores within a single perithecium and a single ascus, no dwarf spores or giant spores have been found in this species. The characteristic pits of the ascospore walls which place this fungus in the genus *Gelasinospora*, can be seen best just before the spores mature while they are changing from yellow to light brown in color (FIG. 6). In the mature spores the wall is so opaque that the pits are virtually invisible.

The ascospores are considerably smaller than those of *Gelasinospora cerealis*, the other species with octosporous asci in this genus. No apiculate spores such as found by Dowding (1) in *Gelasinospora cerealis* have been found in the new species.

The ascospores show two nuclei when stained with propionocarmine when still young (FIG. 6). Young ascospores with single nuclei also have been found. The nuclear situation appears to be similar to that in the other two species of this genus, the two nuclei in the mature spore probably being sister nuclei. Extensive cytological investigations have not been carried out as yet.

## ACKNOWLEDGMENTS

We wish to express our sincere appreciation to the All College Research Committee of Michigan State College, for a grant, a portion of which was used to support this investigation; to Dr. E. Silver Keeping for supplying cultures of *Gelasinospora cerealis* and *Gelasinospora tetrasperma* for comparative studies; to Dr. L. S. Olive for helpful suggestions on the use of the propiono-carmin smear technique, and to Drs. B. O. Dodge and E. A. Bessey for valuable suggestions, the former through correspondence, the latter on his frequent visits to our laboratory. Thanks are also due to Dr. E. A. Bessey for criticizing the manuscript. Our gratitude also goes to Dr. William M. Seaman of the Michigan State College Department of Foreign Languages for correcting the Latin description and for his helpful suggestions in the selection of some descriptive words.

After this article was submitted for publication, the December 1949 number of the *Supplément Colonial à la Revue de Mycologie* was received. In an article entitled *Quelques Ascomycètes du Congo*, Dr. and Mme. Claude Moreau describe and figure (pp. 53-55) *Gelasinospora calospora* (Mouton) nov. comb. This is presumably a homothallic species which closely resembles our fungus in its morphology.

Correspondence with Dr. Roy F. Cain of the University of Toronto has also revealed that he has an article in press (*Canadian Journal of Research*) in which *Gelasinospora adjuncta*, consisting of two strains, is being described.

A comparative study of these organisms with ours is necessary to establish the validity of *Gelasinospora autosteiira*.

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#### EXPLANATION OF FIGURES

FIG. 1. Ten stages in the germination of an ascospore, drawn at approximately half-hour intervals as follows: A—3:30, B—4:00, C—4:30, D—5:00, E—5:30, F—7:05, G—7:40, H—8:15, I—8:40, J—9:12. All camera lucida ( $\times 375$ ).

FIG. 2. Photomicrograph of ascospore 22 hours after planting ( $\times 150$ ).

FIG. 3. Photomicrograph of ascospores within asci, the latter spread out after crushing a perithecium under a cover slip. Note prominent ascial pores, aborted spores ( $\times 250$ ).

FIG. 4. Corn meal agar cultures of: A. *Gelasinospora autosteira* A and B strains with perithecia produced all along the line of contact between the two mycelia; B. A and B strains with perithecia produced first in two groups near the margin of the Petri dish at the point of contact of the two mycelia, and subsequently scattered between these two points; C. *Gelasinospora cerealis* showing numerous perithecia produced by a monosporous culture; D. *Gelasinospora autosteira*, producing no perithecia when two monosporous isolates of the same strain are grown together; E. Monosporous isolate of *Gelasinospora autosteira* producing no perithecia. All cultures of the same age, one month old.

FIG. 5. Perithecium of *Gelasinospora autosteira* on stem of *Tillandsia* ( $\times 100$ ).

FIG. 6. Ascospores of *Gelasinospora autosteira* at various stages of maturity stained with propiono-carmin. On the left, several ascospores showing two nuclei each. Lower margin, one ascospore with a single nucleus. On the right, ascospores with pitted walls ( $\times 500$ ).

## URNULA CRATERIUM IS POSSIBLY THE PERFECT STAGE OF STRUMELLA CORYNEOIDEA

ROSS W. DAVIDSON<sup>1</sup>

(WITH 3 FIGURES)

In the Civilian Conservation Corps program in timber stand improvement work, which began in 1933, the Division of Forest Pathology became increasingly interested in cankers of hardwoods. The interest at that time was mainly from the standpoint of formulating methods of disease control. Cultures were obtained of a number of the more important canker fungi such as species of *Strumella*, *Nectria*, and *Eutypella* and some have been maintained in the stock culture collection of the Division of Forest Pathology at Beltsville, Maryland, since that time. *Strumella* canker (caused by *S. coryneoidea* Sacc. and Wint.) has long been regarded as one of the more important defects of species of oak and several other hardwoods (1, 3, 4, 6, 8). The perfect stage of the fungus causing this canker was not known but has been the subject of speculation among forest pathologists. The perfect stage has not been taken into consideration in formulating control practices and this may be illustrated by the following quotation from Boyce (2): "If cankered living trees with dead or likely-to-be-killed branches or sprouts adjacent to cankers cannot be removed, the branches or sprouts should be knocked off. . . . Furthermore, all standing dead trees with bark on should be knocked down." This was based on the observation that conidia of the fungus develop in abundance on infected dead standing trees although it has not been demonstrated that conidia are the source of infections. In fact, none of the investigators has been able to germinate conidia on artificial media (1, 6, 8).

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## EVIDENCE OF A PERFECT STAGE OF STRUMELLA

In December 1948, peculiar fingerlike fungus growths (FIG. 1A) on an oak log were called to the attention of the writer.<sup>2</sup> These were on oak logs about 5 to 6 inches in diameter which had been cut during 1946 in clearing an area to be used for a golf course near Fairfax, Virginia. The growths were quite numerous and occurred on the under side of the logs where they had been in contact with the damp soil or leaf litter. They were dark gray to black, with a white powdery outer surface, about one inch long, cylindrical, rounded at the tip, and occasionally slightly enlarged at the tip end. They were about the diameter of a pencil. On sectioning they were found to be hollow above and with an immature hymenial layer on the inside, suggesting an immature disco-mycete. The bases of the immature fruiting bodies were surrounded by a black hairlike mass of mycelium (FIG. 1A).

Isolations from the inner bark tissue on which the fungus was growing yielded in 10 cases out of 12 what appeared to be cultures of *Strumella coryneoidea*, as shown by comparison with stock cultures of *Strumella* from oak cankers that had been maintained in the culture collection for about 15 years.

Additional sections of oak logs containing immature fruiting bodies of the fungus were collected near Fairfax, Virginia, in February 1949, and kept outdoors. On March 31, 1949, several of the immature sporophores had enlarged at the tip and started to split open in a stellate manner (FIG. 1B). No mature asci were present on this date and they did not mature when placed in a damp chamber in the laboratory for further development (FIG. 1C). However, pure cultures were readily obtained by picking out small bits of the hymenial tissue.

On April 18, 1949, several opened apothecia were again brought into the laboratory, but asci were immature, showing only in a few cases eight differentiated nuclei. On April 25, two apothecia were collected from the logs and taken to the laboratory. These contained a few apparently mature ascospores so that the sporophores were definitely identified as *Urnula craterium* (Schw.) Fr. On

<sup>2</sup> Mr. Walter Roney, formerly of the Division of Forest Pathology, called attention to this fungus and assisted in collecting the material used in this study.

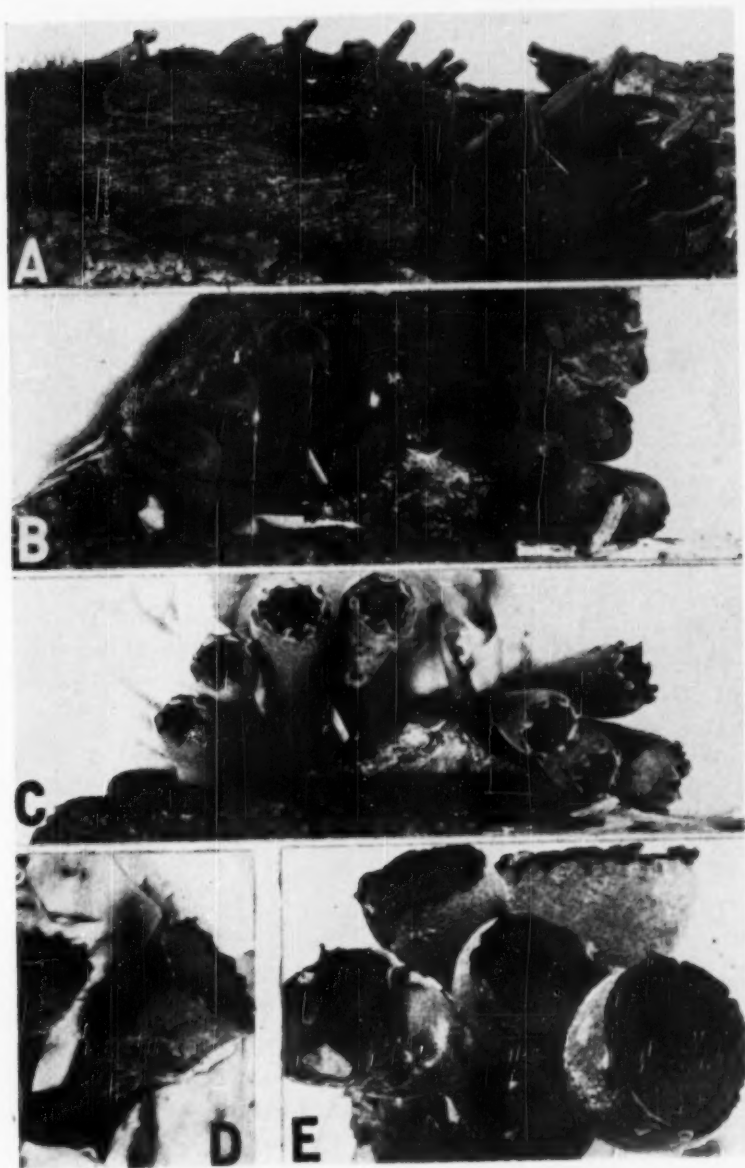


FIG. 1. Developmental stages of *Urnula craterium*.

April 26, two apothecia were quite wide open (FIG. 1D), and a deposit of ascospores was obtained. Cultures from the ascospores were made on malt and cornmeal agar media (FIG. 1D).

Within the following few days in late April, additional apothecia were collected in mature condition (FIG. 1E). When placed over Petri dishes containing cornmeal agar, ascospores were deposited in abundance. The spores were sometimes shot out in scattered groups of eight or only one or two spores at a time. Cultures were readily obtained.

Germination occurred from one end in 3 to 4 hours in 90 to 100 per cent of the ascospores shot from the apothecium. Sometimes a second germ tube grew from the opposite end within about 24 hours after the spores had been deposited on agar. Subsequent growth of germ tubes was very rapid. In a 24-hour period the germ tubes were many times the length of the spore.

#### CULTURES OF *URNULA CRATERIUM* AND *STRUMELLA*

Cultures of *Strumella coryncoidea* as obtained from cankers are rather distinctive and differ considerably from cultures of any other fungus which the writer has studied. They grow rapidly, forming a light gray dense mat on malt agar. Old cultures develop dark, sometimes black areas. In test tube cultures rather dense black masses of fungus tissue develop between the glass and the agar substratum (FIG. 2B), as described by Bidwell and Bramble (1). These appear to be somewhat comparable to the masses of fungus tissue forming the pustules that break through the bark—serving as pressure pads. Those that develop in test tubes are composed of masses of coiled segments of dark black, encrusted hyphae 3 to 5  $\mu$  in diameter.

Cultures obtained from the bark on which immature fruit bodies of *Urnula* were growing and tissue isolations from the nearly mature sporophores were similar to cultures of *Strumella* in growth rate on malt agar, malt plus gallic acid, and malt plus tannic acid. They were slightly more zonate and the mycelial mat on malt was a little darker than those of the old stock cultures. Cultures from single ascospores were lighter and less zonate, or nearly the same as the cultures of *Strumella*. This variation in cultures from dif-

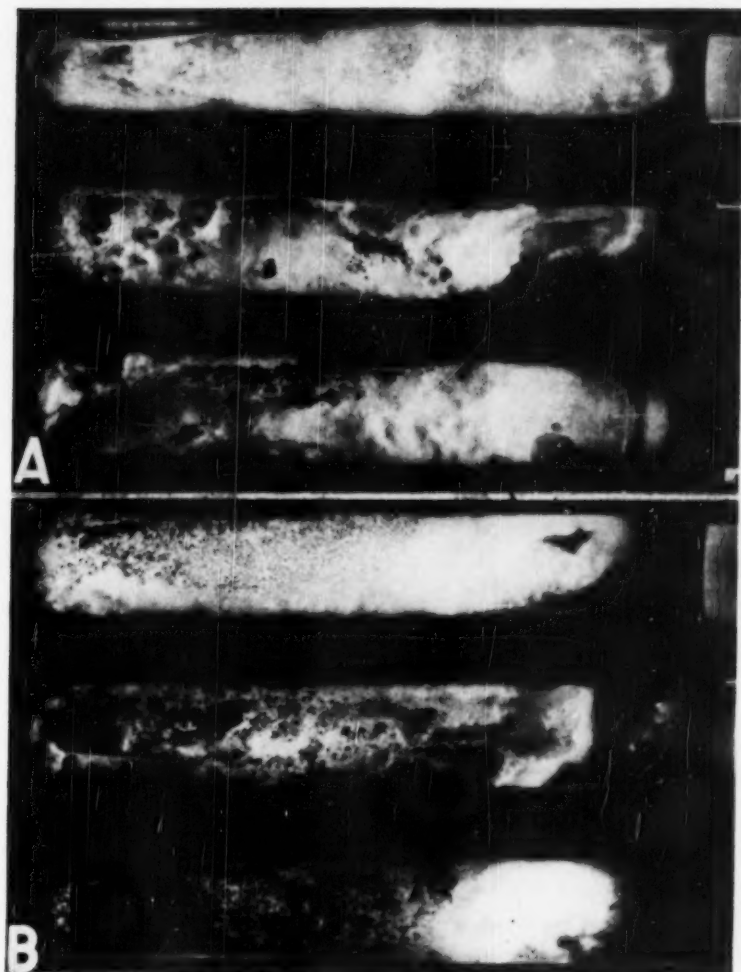


FIG. 2. Cultures of *Urnula* and *Strumella*.

ferent sources is believed to be no greater than would be expected with cultures of a single species from different sources. The *Urnula* cultures also developed the black stromatic masses in test tube cultures (FIG. 2A).

The growth and reactions of *Strumella* on media containing gallic and tannic acid were recorded in a previous paper (5). The

growth and reaction of cultures of *Urnula craterium* on these media are very similar. Growth rate of both fungi on two per cent malt agar at ordinary room temperature of 24° C. is about 50–60 mm. in diameter, in one week. On gallic acid medium under similar conditions it is 0–20 mm., on tannic acid about 20–30 mm. Both fungi give a strong reaction on gallic and tannic acid media.

Conidia of *Strumella* have never been observed to develop in pure cultures so none would be expected under ordinary culture conditions. They might be induced to form if different types of substrata were used.

#### URNULA IN AREAS WHERE STRUMELLA CANKERS ARE NUMEROUS

The only sporophores observed in the spring of 1949 were all from oak logs collected at the one place near Fairfax, Virginia. A search at other places near the District of Columbia, especially at Beltsville, Maryland, failed to locate any additional specimens. *Strumella* cankers are not known to be common in this general area.

On December 8, 1949, additional material of *Urnula* was collected on a canker control plot near Oakland, Maryland. In this area *Strumella* cankers are abundant on oak reproduction. Cankered trunks that had been felled or that had broken over and remained on the ground for several years, contained abundant young fruits of *Urnula*. These were about the same as those collected near Fairfax, Virginia, but were mostly younger and somewhat more cone-shaped, with a broad base, and tapering to a rounded tip (FIG. 3). From this material it is evident that the fruit bodies start to develop in the fall, probably in October or early November. They appear to develop only on trunks that have been lying on the damp ground for some time. It is believed that *Urnula craterium* is a common fungus in other localities where *Strumella* cankers are prevalent.

In a recent paper Nannfeldt (7) described a new species, *Urnula hiemalis*, which has slightly smaller ascospores and shorter stipes than *U. craterium* and grows on grassy slopes or sparse lawn, apparently not attached to sticks. The new species develops during the winter in which respect it is probably similar in habit to *U. craterium* except that the apothecia may open up earlier. Both

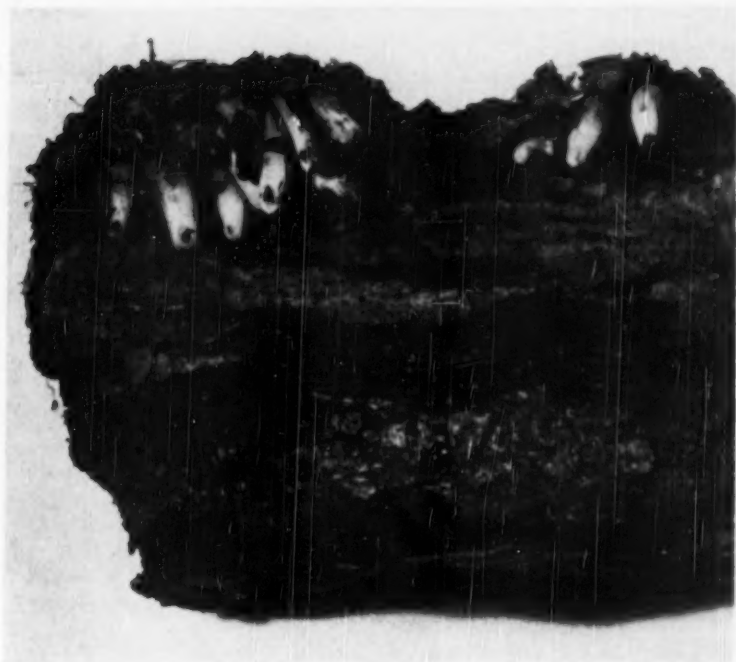


FIG. 3. Young sporophores of *Urnula* photographed on Dec. 9, 1949.

species have the coarse black hairlike mat at their base. *U. craterium* is reported as very rare in Europe.

#### CONCLUSIONS

Present observations and studies strongly suggest that *Urnula craterium* is the perfect stage of the fungus causing *Strumella* cankers. Cultures of *U. craterium* have been compared with several cultures of *Strumella* which were originally isolated from cankers. These cultures were found to be very similar. Sporophores of *Urnula* start to develop in the fall (October or November) and mature by the latter part of April or the first part of May. Ascospores germinate in a few hours when deposited on nutrient agar media. Sporophores of the perfect stage have been observed to develop only on wood in contact with the damp soil and leaf mold.

They were found in abundance in an area where *Strumella* cankers are common.

This evidence suggests that in timber stand improvement work designed to reduce canker incidence, trees with *Strumella* cankers should not be cut down and left on the ground.

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#### DESCRIPTION OF ILLUSTRATIONS

FIG. 1. *Urnula craterium* in several stages of development. A. Young sporophores collected in December 1948. B. Sporophores, collected March 31, 1949, just starting to split open at the tip. C. The same sporophores shown in B, after being held in the laboratory for several days. Asci were present but ascospores had not developed. D. Sporophores collected April 25, 1949. A few mature ascospores were present at that date. E. Mature sporophores collected April 28, 1949.

FIG. 2. A. Cultures of *Urnula craterium* at different stages of development. B. Stock cultures of *Strumella coryneoides* at different stages of development, isolated from cankers.

FIG. 3. Young sporophores photographed December 9, 1949, in fresh condition on cankered oak stem that had been lying on the ground, near Oakland, Maryland.

## NEW AND NOTEWORTHY LICHENS FROM MT. RAINIER NATIONAL PARK

HENRY A. IMSHAUG

(WITH 2 FIGURES)

During the summer of 1948 the writer was fortunate in having the opportunity to assist Dr. A. H. Smith in a mycological survey of Mt. Rainier National Park, in the state of Washington. A number of papers dealing with the new and noteworthy fungi collected there have already appeared, and others are in preparation. The purpose of this paper is to discuss some of the new or interesting macrolichens. The microlichens will be considered at a later date.

I wish to take this opportunity to express my appreciation to Dr. E. B. Mains, director of the University of Michigan Herbarium, for making this survey possible, as well as for reading the manuscript, and also to Dr. A. H. Smith and J. Hedrick Jones for their advice and suggestions in the course of the collection and determination of the specimens and preparation of the manuscript. I am especially indebted to T. E. Hasselrot, Veli Räsänen, C. W. Dodge, and Ove Almborn for the loan of type material and other critical specimens in the course of this study.

### 1. A CORTICOLOUS *Cetraria* IN THE *islandica*-GROUP

This *Cetraria* was very abundant on the lower branches of small, thicket-forming shrubs, e.g., *Rhododendron albiflorum* and species of *Vaccinium*. Also, it grew equally well on the soil around the bushes, especially in the more open areas at the higher elevations. In the literature this plant has been described both as *C. islandica* mod. *arborialis* and *C. tenuifolia* var. *pseudoislandica* f. *septentrionalis*.

Although this lichen differs markedly from both *C. islandica* and *C. crispa* (= *C. tenuifolia*), previous workers have considered it to be a modification resulting from its corticolous habitat. Th.

Fries (1871, p. 98) observed that small forms of *C. islandica* were sometimes to be found on wood and so little significance was attached to the American specimens.

The material found on Mt. Rainier was fruiting abundantly and large collections were made. The apothecia are marginal, at times almost stipitate, rather than subterminal on dilated apices as in *C. islandica* and *C. crispa*. The apothecial margins are not ciliate. These characters, together with the almost plane, nitidous laciniae, the complete lack of laminal pseudocyphellae, and the chemical reactions (Pd—, I—), clearly show, in the writer's opinion, that this plant cannot be placed as a variety or form under either *C. islandica* or *C. crispa*. Also, the Mt. Rainier material clearly shows that these differences are not due to a corticolous habitat, i.e., the lichen was equally abundant on shrubbery and on the ground, without any apparent morphological change. When lignicolous, however, it is fastened to the substratum by black, adhesive discs which develop from the tips or the margins of the laciniae which come in contact with the bark. Growth of the lichen creates a tension on the laciniae fastened to the bark and eventually the adhesive discs are pulled away from the substratum. These discs persist on the elevated laciniae and are characteristic of the species when growing on bark.

It was, of course, desirable to compare this material with the Swedish specimens of *C. islandica* mentioned by Th. Fries and others as occasionally growing on wood. For this purpose, Dr. T. E. Hasselrot, of the Riksmuseum, Stockholm, was kind enough to examine the more than 900 envelopes of this group in that herbarium and to forward for study the twenty-five corticolous specimens, collected by Efr. Eriksson, which he found. Most were on stems of *Juniperus communis*, but some were from spruce and pine. Twenty-one were typical *C. islandica*, whereas four were typical *C. crispa*. In addition, the writer was able to examine a specimen of *C. islandica* f. *rhododendri* (Britzelmayer, Lichenes exs. aus der Flora von Augsburg, No. 633, Farlow Herbarium, Harvard University), which proved to be typical *C. islandica*. The Mt. Rainier material showed no similarity to any of these corticolous specimens.

It may be added that instances of *C. crispa* growing on wood without any apparent morphological change in the thallus are not entirely unknown in North America, as the writer has collected specimens on low juniper branches and on wood debris along the shores of Lake Michigan and Lake Huron in the state of Michigan.

In view of the information presented here it is concluded that both *C. islandica* and *C. crispa* are able to grow on small stems and branches, stumps, and old wood, without any apparent morphological change, and that the lichen known as *C. islandica arborialis*, which has been viewed as a modification of *C. islandica* due to its corticolous habitat, differs from both *C. islandica* and *C. crispa* by a combination of characters such as the position of apothecia, the lack of laminal pseudocyphellae, and the chemical reactions. Consequently, it cannot be considered as a variety or form under either of these species.

The question of nomenclature merits special consideration. The first published name was *C. islandica* modification *arborialis* (conditional nomination) Merrill. In addition to the peculiar designation, the following facts should be noted: 1) no type specimen was cited; 2) the description was based on two different collections, representing two distinct species, as shown by Howe (1915). Following the International Rules, Merrill's publication of the epithet *arborialis* cannot be considered valid.

The second publication of this name was by Howe (1915). Again, several facts may be noted: 1) the composite nature of Merrill's description was corrected; 2) a type specimen was designated; 3) the rank given to this name remained that of modification. Howe's use of the term modification may be explained by the following quotation (1915, p. 219): "It is evidently a modification as Mr. Merrill called it, caused by the corticoline substrata, a more or less accidental result of environment. . . ." He concludes by saying "further material of this interesting modification may prove to argue its acceptance as a variety or even species." In the writer's opinion this cannot constitute valid publication as Article 37 of the International Rules (Camp *et al.*, 1947) says "A name of a taxonomic group is not validly published unless it is definitely accepted by the author who published it. A name pro-

posed provisionally (nomen provisorium) in anticipation of the eventual acceptance of the group or of a particular circumscription, position or rank of a given group, or merely mentioned incidentally is not published."

The use of the combination *C. islandica* var. *arborialis* by Zahlbruckner (1930, p. 333) without any comment or without citing a particular description does not seem valid in view of the fact that Merrill's original description was a composite, based on two distinct species.

Finally, the epithet *septentrionalis*, used at the form level by Räsänen, is not available for use at the species level, due to the existence of *Cetraria septentrionalis* (Nyl.) Almqu., a synonym of *C. chrysanth* Tuck.

In view of these considerations it is necessary to propose a new name for this species. One which indicates its subalpine habitat is deemed suitable.

***Cetraria subalpina* sp. nov. FIG. 1**

*C. islandica* m. *arborialis* Merrill, Bryologist 9 (1): 4. 1906.

*C. islandica* var. *arborialis* Zahlbr., Cat. Lich. Univ. 6: 333. 1930.

*C. islandica* var. *pseudoislandica* f. *septentrionalis* Räsänen, Ann. Mo. Bot. Gard. 20: 9. 1933 (type seen).

Thallus laciniato-lobatus, laciniis cartilagineis, procumbens ad erectis, obtortis, irregulariter ramosis, superne et subtus glaber, nitidus. Margo laciniarum fere spinis. Color pallidus, pallide castaneo-umbrinus, olivaceo-umbrinus vel subfuscus. Pseudocyphellae longae et angustae, in marginibus. Rhizinae desunt. Soredia et isidia desunt. Apothecia 2.5 mm. lata, in marginibus laciniarum; margo apotheciarum non ciliatus. Sporae subglobosae, diam. 4.3-6.4  $\mu$ . Thallus extus et intus K—, K(C)—, C—, J—, Pd—. Specimen typicum in ramis, 6000 ft., Mt. Rainier National Park, Washington, U. S. A., Imshaug 1876, in Herb. Mich.

Thallus composed of densely tangled, twisted, irregularly divided, prostrate to erect laciniae. Laciniae cartilaginous, glabrous, nitidous; plane or with prominent central groove, occasionally with two grooves separated by a central ridge, but not canaliculate-connivent as in *C. crispa*; never expanded, main branches 4.0-6.5 mm. across, tips 1.0-1.5 mm. across; margins  $\pm$  spinulose. Color very variable, pallid, greenish-olivaceous, olivaceous-fuscescent, earth-brown, rich chestnut brown, or combinations of these; lower

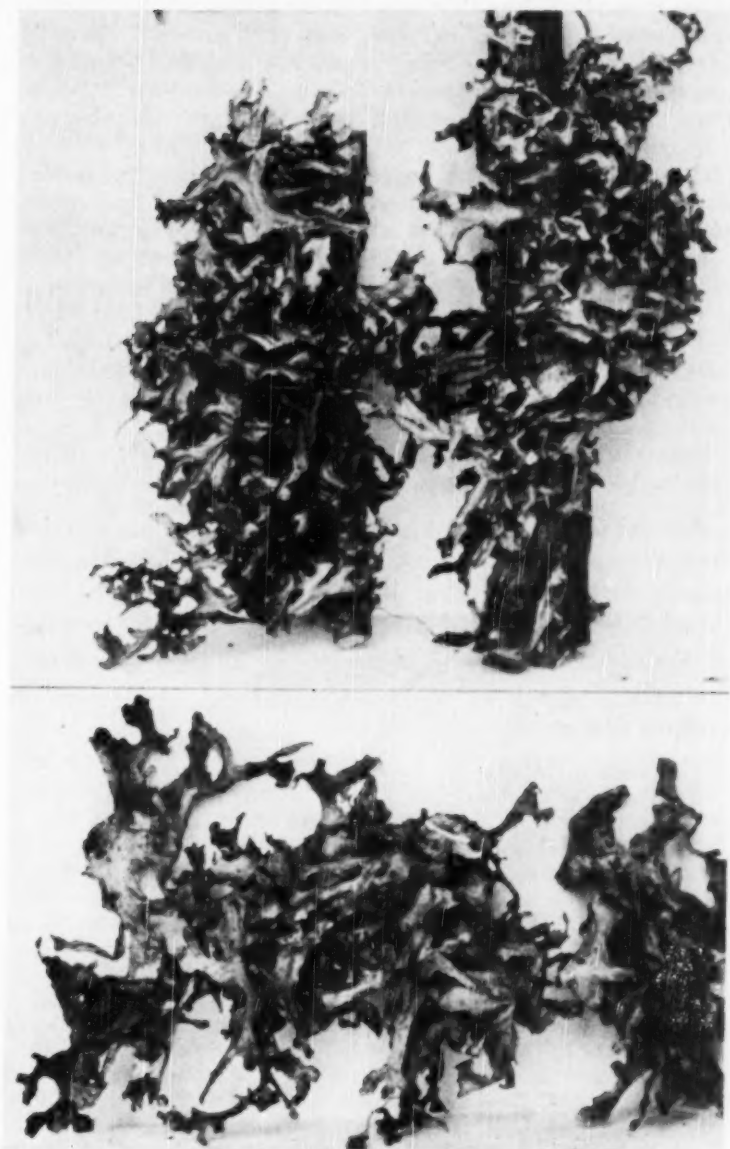


FIG. 1. *Cetraria subalpina*. Upper, corticolous; lower, terricolous.

surface generally lighter than upper surface, often pallid. Pseudocyphellae inconspicuous, marginal, long and narrow. Rhizoids none. Soredia and isidia none. Apothecia marginal, originating from upper surface of laciniae. Disc buff to light brown, 2-5 mm. across. Margin at first prominent, inrolled, at length disappearing;  $\pm$  scalloped; not ciliate.

Thallus 150-220  $\mu$  thick; upper cortex scleroplectenchymatic, uncolored, 25-32  $\mu$ , with cuticle c. 3.2  $\mu$  thick; algae bright green, spherical, 8-11  $\mu$  in diam., in  $\pm$  continuous zone 30-45  $\mu$  thick, also scattered in medulla; medulla of loose, pachydermatous hyphae, colorless, white in reflected light, 50-70  $\mu$  thick; lower cortex similar to upper cortex. Hymenium colorless, yellow-fuscescent above, 50-80  $\mu$ ; asci subcylindric to narrowly clavate, 35-52  $\times$  8.4-12.7  $\mu$ ; spores 8, subspherical to irregular, 4.3-6.4  $\mu$  across; paraphyses simple, unbranched, c. 2.2  $\mu$  across, apices not capitate; hypothecium colorless, 68-82  $\mu$ .

Chemical reactions: Thallus Pd—, J—, K—, K(C)—, C—; medulla Pd—, K—, K(C)—, C—, J—; asci J + (blue) above.

Habitat: On branches of small shrubs, e.g., *Vaccinium* spp. and *Rhododendron albiflorum*, and on the soil beneath them. Also, occasionally on shaded, mossy banks.

Distribution: In the Olympic Mountains and Cascade Mountains of Washington (U. S. A.), in the Selkirk Mountains of British Columbia (Canada), in the Northern Rockies (Canada), and in northern Alaska.

Specimens seen: Alaska: Pt. Barrow (Lehnert's collection, U. of M.<sup>1</sup>). British Columbia: Glacier—(Fink 4916, U. of M.), beside Great Glacier, 6500 ft. (Fink 5909, U. of M.), Hermit Mt., 6000-7000 ft. (Fink 5768 & 5797, U. of M.); Golden—(Kujala, Herb. Mo. Bot. Gard. No. 69142—type specimen of *C. tenuifolia* var. *pseudoislandica* f. *septentrionalis* Räsänen); Yoho Nat'l Park—Yoho River below Lake Duchesnay (Imshaug 6728). Alberta: Jasper Nat'l Park—ridge opposite Angel Glacier on Mt. Edith Cavell, 6200 ft. (Imshaug 7033 & 7037). Washington: Olympic Mts.—Humes Glacier, 5000 ft. (Frye 14, U. of M.), Hurricane Ridge, 5500 ft. (Smith 2358, U. of M.), Crystal Ridge (Smith 14980, N. of M.), Sol Duc Hot Springs District (Eyerdam 1553, U. of M.); Mt. Rainier Nat'l Park—(Plitt, Aug. 1918, U. of M.), Longmire Springs (Fink 4801, U. of M.), Hidden Lake Trail, 5500-6000 ft. (Imshaug 261, 263 and 267), summit of Yakima Peak, 6200 ft. (Imshaug 484), Kotsuck Creek, 4500 ft. (Imshaug 131), eastern slope of Cowlitz Divide, 3000-4000 ft. (Imshaug 155), Eagle Peak Trail (Imshaug 1567), summit of Chutla Peak, 6000 ft. (Imshaug 1623), Nisqually River Mining Camp, 2900 ft. (Imshaug

<sup>1</sup> U. of M. = University Herbarium, University of Michigan.

1385), Reflection Lake, 4861 ft. (Imshaug 1074), Lake George Trail, 4000 ft. (Imshaug 1940), Mt. Wow, 6000 ft. (Imshaug 1876—**type**), Cataract Falls, 4100 ft. (Imshaug 510). Oregon (?):<sup>2</sup> Cascade Mts. (Hall, 1871, Farlow Herb., Harvard Univ.).

2. *Pseudocyphellaria rainierensis* sp. nov. FIG. 2.

Thallus 1–2 dm. latus, fragilis, laciniato-lobatus, pallide virido-cinereus, laciniis (1) 3–8 cm. longis (0.7) 1.5–3.0 cm. latis, imbricatis, marginibus erosis, coralloideoisidiosis, superne plus minusve foveolatus, glaucus, subtus fere ad apicem dense tomentosus, obscure fuscus, apicibus pallidis vel pallidioribus. Pseudocyphellae densae, irregulariter verruciformes, albae. Algae virides, globosae, diam. vulgo 6.4–10.6  $\mu$ . Pycnoconidia bacillaria, recta, 4.2–5.4  $\times$  c. 1  $\mu$ . Apothecia desunt. Thallus K + intense lutescens, K(C)—, C—, J—, Pd—; medulla K—, K(C)—, C—, J—, Pd—. Specimen typicum in cortice, 2400 ft., Mt. Rainier National Park, Washington, U. S. A., Imshaug 598, in Herb. Mich.

Thallus large, 1–2 dm. across, brittle, lacinate-lobate, pale greenish-cinereous. Lacinae (1) 3–8 cm. long, (0.7) 1.5–3.0 cm. broad, imbricate; apices broadly rounded, incised crenulate; margins erose to crenate-lacinulate, bearing abundant, coralloid isidia; surface unevenly impressed to foveolate, glaucous, occasionally with isolated clusters of coralloid isidia (not limited to cracks or other injuries, although especially abundant in such areas), endotrophic cephalodia occasionally visible as low warts above surface. Lower side pale buff to brownish yellow towards center, tomentose to the margin; tomentum usually dense, pale. Pseudocyphellae conspicuous, dense, white, 0.2–0.6 mm., verruciform or flattened, margin indistinct. Pycnoconidangia, when present, prominent, superficial, immersed, with the visible part black. Apothecia not seen.

Thallus 0.3–0.4 mm. thick. Upper cortex 38–50  $\mu$ , yellowish-brown, paraplectenchymatous; cells (KOH) rather indistinctly arranged in c. 6 horizontal rows, 5.3–7.5  $\times$  3.5–4.3  $\mu$ , elongated horizontally, moderately thin-walled. Algal layer 37–45  $\mu$ ; algae green, 6.4–10.6  $\mu$  in diam. Medulla gray, 215–235  $\mu$ ; hyphae  $\pm$  intricate, 3.8–4.2  $\mu$ , leptodermatous, not granular. Lower cortex yellowish-brown, 32–44  $\mu$ , cells similar to those in upper cortex. Rhizinae fasciculate, pale yellow-brown, c. 125  $\mu$  long; hyphae 6.4–9.5  $\mu$  thick, thin-walled, indistinctly septate. Endotrophic cephalodia with blue-green algae. Pycnoconidangia 0.4  $\times$  0.3 mm.; pycnoconidia bacillar, straight, 4.2–5.4  $\times$  c. 1  $\mu$ .

Chemical reactions: Thallus K + (intense yellow), K(C)—, C—, J—, Pd—; medulla K—, K(C)—, C—, J—, Pd—.

<sup>2</sup> The word Oregon on the label probably refers to the Oregon Territory and the specimen could have come from what is now the state of Washington.

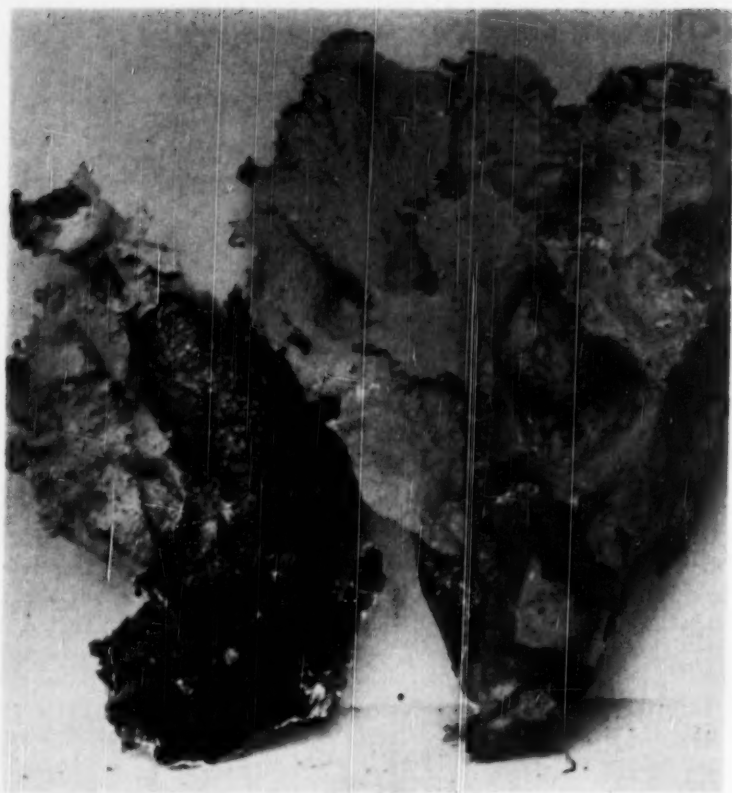


FIG. 2. *Pseudocyphellaria rainierensis*.

Habitat and distribution: On bark of alder and fir at elevations of from 2300–2700 ft., Ohanapecosh River above Panther Creek, Imshaug 598—**type**; Ohanapecosh River above Laughing Water Creek, Imshaug 722; Nisqually River, Imshaug 1362; Tahoma Creek, Imshaug 1238.

Discussion: This species, in many respects, reminds one of *Lobaria oregana*, but differs principally in the presence of pseudocyphellae on the under surface, although the color, texture, and KOH-reaction are also at variance. Points of similarity are the large size,  $\pm$  foveolate upper surface, extreme brittleness, and coralloid isidia. Also, the endotrophic cephalodia, with blue-green algae, are similar to those described for *L. oregana* by Schneider

(1897, p. 58). The large size of *Pseudocyphellaria rainierensis* and the diagnostic characters mentioned above prevent its confusion with any other North American lichen. *Cyanisticta Hookeri* var. *septentrionalis* Räsänen (1933, p. 17) appears similar from its description. A study of the type specimen, however, showed that it differs in its much smaller size and in the questionable presence of white pseudocyphellae, as well as in the nature of the algae.

*Pseudocyphellaria rainierensis* should be looked for in the Olympic Mountains of Washington, U. S. A., and the Selkirk Mountains of British Columbia, Canada. It will most likely be limited in its distribution to these regions of heavy precipitation in our Pacific Northwest.

3. *ALECTORIA ALTAICA* (Gyel.) Räs., Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo 12 (1): 34. 1939.

*A. spinulosa* Ahlner, Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo 9 (1): 35. 1937 (nomen nudum).

*A. karelica* Räs., Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo 12 (1): 34. 1939.

*A. altaica* var. *spinulosa* Räs., Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo 12 (1): 34. 1939.

*Bryopogon altaicus* Gyel., Tisia 2: 166. 1937.

Original description: "Thallus erectus, usque ad 4 cm longus, sat opacus, ad basin nigricans, versus apices pallidus K + flavus, C—, KC + flavus dein celeriter evanescens, P + citrinus deindeque rubescens, in partibus nigrescentibus K—, C—, KC—, ramis divaricatis non rectangulis, rariter subrectangulis instructus, sorediosus, sorediis lateralibus, maculiformibus, sat elongatis vel subrotundatis, albidis, maculis sorediorum vulgo latioribus sicut ramis, ca. 0-1:0-0,6 mm magnis. Pseudocyphellae desunt. Sterilis. Species sectionis *Ochroleucae* Gyel.—Similis *Bryop. Berengeriano* (Mass.) Gyel. sed thallus K + et KC +, etc.—U. S. S. R., Si-birien, Altaigebirge, leg. N. N. Lawrow, comm. M. P. Tomin, no. 20 (Typus in hb. mus. Budapest)."

Figures: Acta Phytographica Suecica XIII (1940), Pl. 3.

Discussion: This *Alectoria* has previously been reported from North America only from Maine (Degelius, 1940) and North Carolina (Degelius, 1941). Outside of North America it appears to be widely distributed in boreal regions. Ahlner (1940) reports

it from the Ural Mountains, Siberia, Middle Europe and Scandinavia. In view of the fact that the species of the genus *Alectoria* have been badly confused in North America, the original description of *A. altaica*, generally unavailable to American students, has been reprinted. In all probability this species is widely distributed in boreal North America.

*A. altaica* is well characterized by its erect to short pendulous habit, the dull, bicolored filaments, the soralia, when present, being farinose, the divergent fibrils, and the KOH + (yellow) reaction. *A. nidulifera* (= *A. chalybeiformis* amer. auct., non. *Lichen chalybeiformis* Linn.) is wiry cespitose, lustrous, not bicolored, and the soralia are covered with spine-like isidia. *A. bicolor* is lustrous, bicolored, esorediate and KOH—. *A. jubata* var. *prolixa* is lustrous, pendulous, lacks the divergent fibrils and is KOH—. *A. implexa* is lustrous, pendulous, pale, lacks the divergent fibrils and is KOH + (yellow).

The Mt. Rainier specimen is sorediate and sterile. It was collected on the bark of *Abies* along the Nisqually River, at an elevation of c. 2700 ft., Imshaug 1364.

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## A NEW SPECIES OF DERMATOCARPON

HENRY A. IMSHAUG

(WITH 1 FIGURE)

During the summer of 1948, while travelling through Yellowstone National Park the writer collected an interesting *Dermatocarpon*. This lichen formed loose, subspherical mounds on soil in a sage brush desert association near the northeast entrance to the park. The unattached mounds gave the appearance of being able to drift with the wind in dry seasons, thus resembling the familiar tumbleweed. In this habit they remind one of *Parmelia molliuscula* and *Lecanora Haydenii*, both characteristic of arid regions of Western United States. *Cetraria islandica* f. *vagans*, described from the desert regions of Siberia by Savicz (1911, p. 51), is also similar in that it forms loose, drifting, spherical mounds.

While examining the collections of *Cornicularia* in the Farlow Herbarium, Harvard University, the author was surprised to find a collection of this *Dermatocarpon* from Mt. Aylmer in the Canadian Rockies. It was distributed by Macoun as No. 105 of Canadian Lichens. Howe (1915, p. 225) says of this number: "appears to be abortive or degenerate material" of *Cetraria Richardsonii*. Although Howe locates Mt. Aylmer in British Columbia, the only Mt. Aylmer the writer is able to locate is in Alberta, on the eastern boundary of Banff National Park, just north of Lake Minnewanka.

An epithet indicative of its drifting habit is deemed suitable for this new species.

### *Dermatocarpon vagans* sp. nov. FIG. 1

Thallus foliaceo-fruticosus, iteratim laciniatus, laciniis primariis 3-6 mm. latis, convolutis, apicibus tenuissimis, irregulariter ramosis et crispatis. superne plus minusve glaber, pruinosis, siccus spadiceus, madefactus virescens, subtus obscurus vel ater, papillosus. Perithecia immersa, ostioliis nigris. Sporae ellipsoideae,  $11.0-15.4 \times 6.2-7.5 \mu$ . Thallus et medulla K—, K(C)—, C—, J—, Pd—; gelat. hym. J + obscure caerulescit. Specimen typicum in solo, Yellowstone National Park, Wyoming, U. S. A., Imshaug 15, in Herb. Mich.

Thallus large, 2-4 cm. across, foliose-fruticose, forming subspherical, unattached mounds on arid soil. Thallus irregularly and deeply lacinate, not in one plane, laciniae projecting at various angles; ends of main laciniae repeatedly dissected and the many lacinulae strike out at all angles, resulting in small tight balls at ends of main branches. Laciniae convolute, main divisions 3-6 mm. across (dry), apices c. 0.5 mm. Upper surface glabrous to minutely rugose; scattered areas white-pruinose; when dry date-brown, green when moistened. Lower surface, rarely visible due to convolute nature of the laciniae, brownish-black, densely papillose. Rhizoids none. Soredia and isidia none. Perithecia numerous, immersed, ostiole black, minute. Pycnoconidangia not observed.

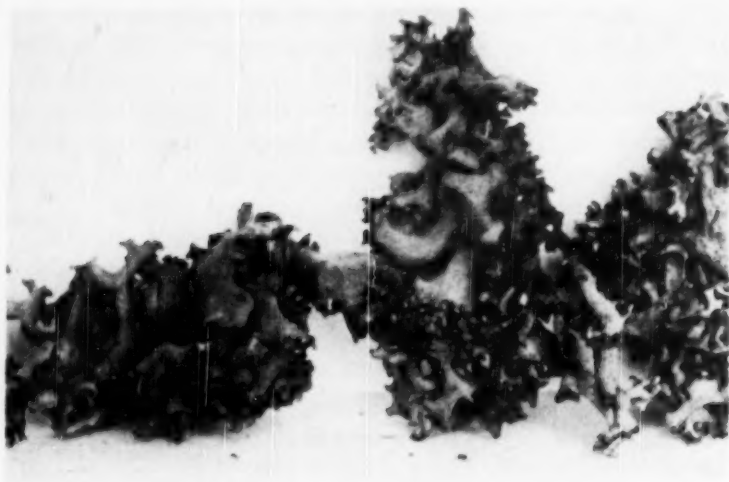


FIG. 1. *Dermatocarpon vagans*.

Thallus 0.4-0.6 mm. thick. Upper cortex 23.5-30.0  $\mu$ , plectynparenchymatic, colorless to pale fuscous above, cells isodiametric, thin-walled, 5.3-8.5  $\mu$ , formed from vertical hyphae. Algal layer 80-120  $\mu$  thick; algae pleurococcaceae, globose, diam. 7.5-10.5  $\mu$ . Medulla lax, 200-325  $\mu$  thick, hyphae intricate, thin-walled, 2.2-2.6  $\mu$ . Lower cortex 40-85  $\mu$  thick, plectynparenchymatic, cells isodiametric, thin-walled, 6.4-10.6  $\mu$ , formed from vertical hyphae, interior cells colorless, outer ones (forming papillae) brown. Perithecia immersed,  $\pm$  globose, pallid to pale rose, diam. 250-300  $\mu$ , wall 40-80  $\mu$  thick, formed from longitudinal hyphae, margin of the

ostiole obscure. Paraphyses indistinct, soon gelatinized. Asci subcylindric,  $54-60 \times 13-15 \mu$ . Spores 8, in two rows, hyaline, ellipsoid, granular, non-septate,  $11.0-15.4 \times 6.2-7.5 \mu$  (length/breadth-coefficient =  $1.7-2.3 \mu$ , average  $2.0 \mu$ ), membrane thin.

Chemical reactions: Thallus and medulla K—, K(C)—, C—, J—, Pd—; gelat. hym. J + (blue).

Habitat and distribution: Growing loose on arid soil, c. 7000 ft., in northeast corner of Yellowstone National Park, Wyoming, June 23, 1948, Imshaug 15—**type**; growing over moss, 8000 ft., Mount Aylmer, Alberta, Canada, Macoun, Canadian Lichens No. 105 (distributed as *Cetraria odentella* (?) Ach.).

Discussion: *Dermatocarpon vagans* is near *D. miniatum* and should be compared with it and the other large-lobed species in the section Entosthelia. *D. miniatum* itself is a widespread and variable plant, characterized by a generally pruinose upper surface, lower surface without rhizoids, and the color unchanging when moistened. It may be monophyllous or polyphyllous, varying from a somewhat imbricate condition to a few medium-sized lobes (var. *complicatum*) or to a compact cushion of many small, densely-congested lobes (var. *panniforme*). These polyphyllous forms are often mistaken for *D. aquaticum*. The lower surface of *D. miniatum* varies in color from yellow-fulvous to fuscous or black. In texture, the lower surface varies from smooth or rugose to reticulate-veined (var. *fulvofuscum*) or may be papillose (var. *papillosum*). It is the papillose forms which present the greatest difficulties. Although not recognized by Fink (1935), *D. miniatum* var. *papillosum* has been reported from Washington by Magnusson (1932) and from New Mexico by Bouly de Lesdain (1942). *D. moulinsii* var. *subpapillosum* Fink apud Hedrick (1933, p. 306) would appear from a study of the type specimen, which is rather meager, to be this variety. *D. vagans*, aside from its habitat and gross appearance, differs from *D. miniatum* in that the color changes to green when moistened.

*D. reticulatum* Magnusson (1932, p. 18), described from the Upper Naches River Region in the Cascade Mts. of Washington, is still incompletely known. It differs from *D. miniatum* var. *papillosum* in its reticulate under surface and in the change of color to green when moistened. Also, the under surface in *D. reticulatum*

seems to be more furfuraceous or granulose than papillose. Specimens of a collection from Tipsoo Lake, Mt. Rainier National Park, near the type locality, made by the writer (Imshaug 220) and determined by Magnusson, show considerable variability in the prominence of the rugae. In some they may even be absent. While the thallus of the type specimen was described as 150–250  $\mu$  in thickness, thinner than in *D. miniatum*, the writer's specimens are 400–600  $\mu$  in thickness. More material is needed of this and the other papillose forms to ascertain the true relationships. *D. vagans* differs from *D. reticulatum* in the lack of prominent rugae and in the more strongly papillose lower surface, in addition to habitat and gross appearance.

The following key to the large-lobed Dermatocarpons of the section Entosthelia summarizes these differences. The distributions given have been compiled from North American material in the University Herbarium, University of Michigan.

- A. Lower surface smooth or wrinkled.
  - B. Thallus green when moistened, not pruinose; generally on wet or inundated rocks.....*D. aquaticum*  
(New England, Que., N. Y., Ont., Mich., Wisc., Ohio, Md., D. C., Ark., Colo., and Wash.)
  - BB. Thallus unchanged when moistened, pruinose; on exposed rocks.  
*D. miniatum*  
(throughout the United States and Canada)
- AA. (see AAA). Lower surface furfuraceous or papillose.
  - C. On rock; thallus  $\pm$  plane, not lacinate.
    - D. Unchanged when moistened; lower surface without prominent rugae.....*D. miniatum* var. *papillosum*  
(Wash., Ore., Idaho, B. C., Manitoba, Mont., and Colo.)
    - DD. Olive-green when moistened; lower surface with prominent rugae.....*D. reticulatum*  
(Washington)
  - CC. On soil, loose; thallus foliose-fruticose, deeply lacinate, convolute.....*D. vagans*  
(Wyoming and Alberta)
- AAA. Lower surface with rhizoids.....*D. moulinsii*  
(Mich., Ont., Colo., Mont., and Wash.)

It may be noted here that the Washington specimens of *D. moulinsii* (Imshaug 178 and 934B) are from Mt. Rainier National Park and are larger than usual for the species (thallus 2.5–7.0 cm.

across, 250–400  $\mu$  thick) and with long (1.5–3.0 mm.), branched, pale yellow-brown to black rhizoids. Also, the lower surface has plates of supporting tissue similar to that in *Umbilicaria Muhlenbergii*. Duplicate material of No. 178, however, was determined as *D. moulinsii* by Magnusson. The large size and the branched rhizoids suggested *D. vellereum* Zschacke (1934, p. 638) but the rhizoids, although branched, are not densely coralloid, as in Elenkin's *Lichenes Florae Rossiae* No. 49a (Univ. of Mich. Herb.).

I wish to take this opportunity to express my appreciation to Dr. A. H. Magnusson, Göteborg, Sweden, for the determination of specimens and to Dr. W. L. White for the use of the facilities of the Farlow Herbarium, Harvard University.

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## SOME LEAFSPOT FUNGI ON WESTERN GRAMINEAE. V<sup>1</sup>

RODERICK SPRAGUE<sup>2</sup>

(WITH 1 FIGURE)

This paper continues a series of discussions and descriptions of fungi found on the grass family in the far west (10).

### VISCOMACULA gen. nov.

Hyphis in maculis in foliis vivis, congregatis in tenues pulvinos, sporulis massas lentas habentibus, hyalinis, brevibus cylindraceis.

Hyphae in spots on living leaves, grouped in extensive shallow pads, spores formed in sticky masses, hyaline, short cylindric. Differs from *Rhynchosporium* in the appreciably thicker mycelial pad and in the gelatinous spore masses.

### Viscomacula aenea sp. nov.

Macula aenea, margine aureo, centro pallido-aeneo, 4-20 × 2-4 mm.; hyphis hyalinis et in foliis et pulvinis tenuibus superficialibus 2-6 cellas crassis; sporulis tenues aeneas lentas massas, ultimo aridas habentibus, hyalinis, brevibus cylindraceis, apice rotundato, raro basi subacuminata, aseptatis, pseudo-multiseptatis (duas vel plures guttulas habentibus), (5.6)6-9(10) × (1.4)1.6-2.0(2.3) μ.

Hab. in foliis vivis *Poa amplae* Merr., inter Loveland Pass et Silver Plume, Colo.; Amer. Bor. Typus est A.S. 20,538. Legit. Sprague, R., Fischer, G. W. et Meiners, Jack P. Aug. 8, 1948.

Spots copper colored, margins yellow, center pale copper colored, 4-20 × 2-4 mm., hyphae hyaline in the foliage and also aggregated on the leaf surface in interrupted shallow gelatinous masses 2-6 cells thick. Spores in small, sticky, copper or carrot colored masses, finally hard and dry, and then obscure; spores borne on ill-formed conidiophores on the flat fruiting areas, short cylindric, ends rounded or sometimes one end somewhat pointed, hyaline or very faintly yellowed, contents biguttulate and giving appearance

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of a wide central septum or rarely 2 such cleared spaces, but no true cross walls seen,  $(5.6)6-9(10) \times (1.4)1.6-2.0(2.3) \mu$ .

Habitat in living leaves of *Poa ampla* along a stream-side meadow below Loveland Pass and about 10 miles up U. S. Highway 6 from Silver Plume, Colo. Type is A. S. 20,538, consisting of about 12 leaves of which only 4 or 5 are good material.

This fungus, collected at an elevation of about 9500 feet, is an active parasite, causing well-defined spots on leaves in part of the somewhat scanty type. It is distinct from anything else which we have seen. The fruiting pad is too well-developed for *Rhynchosporium orthosporum* Caldwell and its apparent sticky or somewhat gelatinous spore mass distinguishes it from the dry spore masses of *Rhynchosporium*. It reminds one of copper spot, *Gloeocercospora sorghi* D. Bain and Edg., but the spores in the Colorado fungus are utterly different. They also differ radically from *Ramulispora*. Its definite moniliaceous nature tends to exclude it from *Gloeosporium*. *G. meinersii* f. *alpina* on *Poa alpina* Merr. has larger spores,  $10-14 \times 3-4 \mu$  (10).

The fungus is apparently an uncommon genus on alpine or sub-alpine grasses. The region, about 10 miles above Silver Plume, Colo., along U. S. Highway 6, was a favorable area for collecting sub-alpine leaf spots.

MATERIAL ON *Deschampsia atropurpurea* collected by Fischer at Logan Pass, Mont., was partly destroyed by insects in the packet before examination was completed. Spores were still present, but no fruiting bodies remained. The spores were of one species. They may represent a fungus of mycological interest because the insect-riddled lesions indicated that the remaining fungus may have originally caused the spot. The spores are pale brown, elliptical-cylindrical,  $8.5-14 \times 4.2-5.7 \mu$  (FIG. 1, A). Further material is needed to determine whether this fungus is only a saprophyte on the insect frass or is a parasitic fungus. If pycnidia were present (*Sphaeropsis*), they certainly were completely destroyed. If it is a member of the micronemae of the Dematiaceae of the Moniliales, a new genus would need recognition.

#### *Stagonospora spartinicola* sp. nov.

Maculis incoloratis v. stramineis, elongatis; pycnidiis obscuris, immersis, sub-globosis, parenchymatis, brunneis, ostiolatis, 150-280  $\mu$  diam.; pycno-

sporulis cylindraceis; constrictis ad septa, utrinque rotundatis, contextu guttulatis, hyalinis, 3-5 septatis,  $40-47 \times 6-9 \mu$ .

Hab. in foliis vivis et emortuis *Spartinae pectinatae* Lk., prope Wilton, N. Dak., Amer. Bor. B.P.I. 80,798, Sept. 14, 1941 (type) et Madison, Wisc. (H. C. Greene) Aug. 13, 1949.

Spots none or when present straw color, drab, faintly buff or light tan, elongate up to  $60 \times 1.5$  mm. diam.; pycnidia immersed but with a relatively large ostiole visible through the slight rupture in the host epidermis, sub-globose to moderately flattened or elliptical in outline, thin walled, parenchymatous, pale brown becoming nearly hyaline in the vicinity of the ostiole,  $150-280 \mu$  diam.; pycnospores cylindrical, constricted at the septa, ends rounded but one more regularly so than the other, contents coarse including a large chlorinous guttula, 3-5 septate,  $40-47 \times 6-9 \mu$ .

On living or dead leaves of *Spartina pectinata* near Wilton, N. Dak. and Madison, Wisc.

This fungus is characterized by the sunken, flattened, pale-colored pycnidia and the cylindrical spores (FIG. 1B) which are larger than those of *St. vexatula* Sacc. to which they show closest relationship. *St. vexatula* on *Phragmites* has spores  $35-38 \times 4.5-5.0 \mu$ , but some are as long as  $55 \mu$ . *Septoria arundinacea* Sacc. has spores  $60-70 \times 5-6 \mu$ . They are longer and narrower than the fungus on *Spartina*. We have been reluctant to consider this fungus as undescribed because of the complex of related forms on coarse marsh grasses. These pycnidial forms involve a consideration of not only *St. vexatula* but nearly twenty other species descriptions. A detailed study of this group indicates, however, that *St. spartinicola* has wider, coarser spores than any of them. The type material from North Dakota, which was apparently saprophytic, is supplemented by a fragment sent by H. C. Greene from Madison, Wisc. The lesions in this material are well delineated and as much as  $60 \times 1.5$  mm. diam. The spores in each collection are very similar, both having large chlorinous guttulae and both varying in number of septa from three to five. The species is likely to prove to be one having seven septa in overwintering material. The spores are cylindrical, but one end tends to be slightly blunter than the other. This aspect, plus strong but variable constrictions, gives the spores a rugged, coarse appearance which I have not been able to completely portray in a simple line drawing.

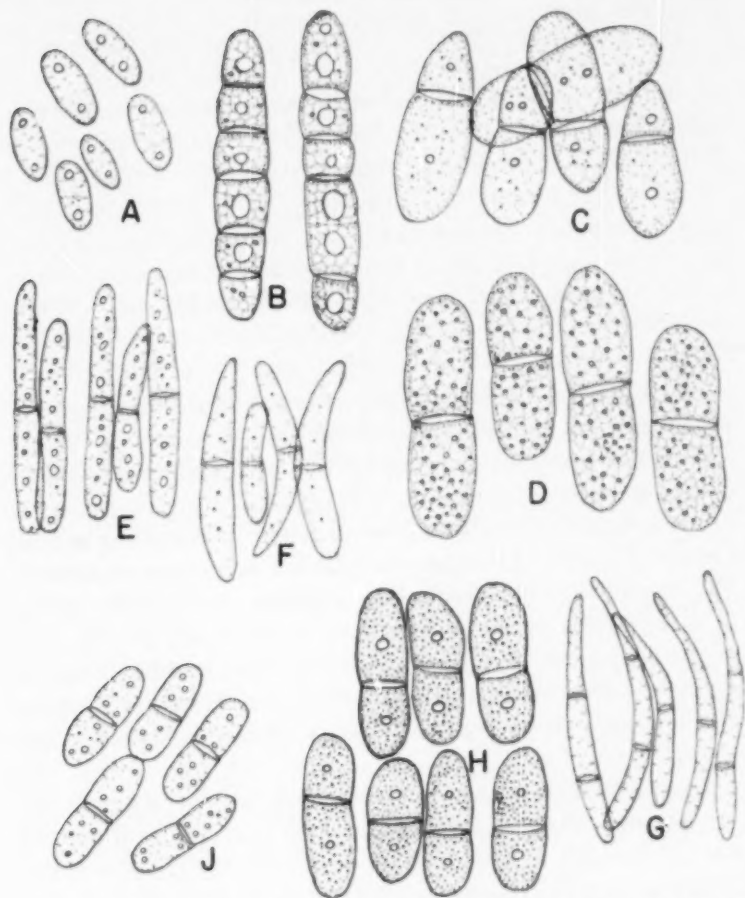


FIG. 1. Spores drawn with the aid of the camera lucida,  $\times 1,000$ . A, Undeterminable, from insect-eaten spots on *Deschampsia atropurpurea*. B, *Stagonospora spartinicola* on *Spartina pectinata*, type. C, *Apiocarpella macrospora* on *Poa ampla*, near Silver Plume, Colo. D, *Stagonospora simplicior* on *Melica bulbosa*, Waterton Lakes Nat'l Park, Alta. E, *Septoria avenae* on *Calamagrostis canadensis*, Baker Hole, Mont. F, *Scaphidium boutelouae* on *Bouteloua curtipendula*, Santa Catalina Mts., Ariz. G, *Phleospora muhlenbergiae*, from type. H, *Stagonospora simplicior* (*Ascochyta* summer stage) on *Stipa columbiana* var. *nelsonii*, Mt. Shasta, Calif. I, *Ascochyta boutelouae* (cf. *Diplodina graminea*) on *Sporobolus airoides*, Pullman, Wash.

## ADDITIONAL SPECIES

*APIOCARPELLA MACROSPORA* (Speg.) Sydow was found on a few leaves of *Poa ampla* Merr. below Loveland Pass on U. S. Highway 6 toward Silver Plume, Colo., by the writer, Fischer, and Meiners (A. S. 20,537). One or two dark brown pycnidia with closed (but developing) ostiolar regions occurred in fawn-colored, elliptical spots on living leaves. The spores (FIG. 1C) differed from saprophytic material of *A. macrospora* on *Stiporyzopsis* from California (9) in that most, but not all, of them were broadly cylindrical and blunt, even at the smaller end. They measured wider ( $8.5\text{--}10\ \mu$ , compared with  $6.6\text{--}8.8\ \mu$ ) but not longer ( $21.4\text{--}26\ \mu$ , compared with  $21\text{--}26\ \mu$ ) than the California collection. Since some of the Colorado spores were pointed at the smaller end and typical of *A. macrospora*, it is questioned if this collection warrants separating from the California specimen previously discussed.

*STAGONOSPORASIMPLICIOR* Sacc. and Berl. is not uncommon as a parasite of *Stipa* spp. and other grasses in western mountains. The fungus on *Stipa* usually has 1-septate, short, thick, spores (FIG. 1D) which we believed might be *Ascochyta stipae* Died. On comparing further, it became evident that Diedicke (2) illustrated a different fungus. His fungus had thick-wall spores, whereas, *St. simplicior* has thin-wall spores and a strong constriction at the central septum.

The material on *Stipa* was arranged for separation from the species proper and a description prepared. Further study showed that the parasitic summer material developed shorter spores (FIG. 1D), but all gradations were found. It is apparent that *St. simplicior*, *St. simplicior* var. *andropogonis* Sacc. and our material on *Stipa* and other mountain grasses can just as well be grouped together. It will be well, however, to publish an English description of the summer developing phase on *Stipa*, since it differs somewhat from the original description of the species:

Spots maroon to vinaceous, sub-circular, prominent but scattered on the leaves, centers of spots paler, finally straw color; pycnidia few, large, globose, erumpent, golden-brown,  $150\text{--}200\ \mu$  diam.; spores broadly cylindrical, ends bluntly rounded, strongly con-

stricted at the central septum, sometimes each half again partially divided by developing septum, guttulate, 1-septate phase  $17.5\text{--}26 \times 6.5\text{--}9.5 \mu$ , 1- to 3-septate phases occur on dying leaves,  $24\text{--}31 \times 9\text{--}11.0 \mu$ , hyaline. Differs from winter material and from var. *andropogonis* in having less robust spores.

Hab. in living or necrotic leaves of: *Stipa columbiana* Macoun, Medicine Bow Nat'l For., Wyo. (A. S. 20,153); *S. columbiana* var. *nelsonii* (Scribn.) Hitchc., Mt. Shasta, Calif., Utah; *S. lemmonii* (Vasey) Scribn., Malheur Nat'l For., Oregon; *S. lettermanii* Vasey, Skyway, Colo.; *S. occidentalis* Thurb., 10 miles n.w. of Sisters, Oregon (A. S. 20,511); *S. viridula* Trin., North Dakota; *S. williamsii* Scribn., Medicine Bow Nat'l For., Wyo. (A. S. 20,526); *Stiporyzopsis bloomeri* (Boland) Johnson, Mt. Whitney, Calif.

While the material on *Stipa*, which was found on living leaves, is typically that of a large-spored species of *Ascochyta*, we have found spores in pycnidia on dead leaves of *Stipa* that are longer, broader, and with 3 septa forming. These spores are very similar to immature ones of *Stagonospora simplicior*. *St. simplicior* is a common saprophyte on a number of grasses (10) but is not infrequently now found to attack healthy plants. Greene has collected material of *St. simplicior* f. *andropogonis*, on several hosts from Wisconsin, that appeared to be actively parasitic. The living leaves harboring the *Ascochyta* phase of the *Stipa* collections have very evidently been invaded by the fungus. Saprophytic material, however, occurs on darker dead leaves in *Stipa columbiana* and *S. lettermanii*. The pycnidia on *S. lettermanii* contain spores  $24\text{--}30 \times 9.0\text{--}10.8 \mu$ . Those on *S. columbiana* have very similar spores  $24\text{--}31 \times 9\text{--}11 \mu$ .

*Stagonospora simplicior* on *Melica bulbosa* (FIG. 1D) was growing with *Septoria nodorum* Berk. in tawny spots on living leaves and sheaths collected by G. W. Fischer at Waterton Lakes Nat'l Park, Alta., Aug. 7, 1949 (A. S. 20,504). While *S. nodorum* was more abundant in the lesions than the *Stagonospora*, both were apparently parasitic. The spores of the *Stagonospora* were coarsely cylindrical, strongly constricted at the first, later at the third septum, somewhat blunted at the ends, the contents coarsely granular but hyaline,  $26.8\text{--}36 \times 9.8\text{--}12.8 \mu$  (FIG. 1D). The pycnidia were much larger than those of *S. nodorum* (about  $150\text{--}180 \mu$  as contrasted with

90–140  $\mu$ ), but otherwise they were similar in appearance in the spots. The *Stagonospora* spores, however, did not readily ooze from the pycnidia as did the much smaller and maturer *Septoria* spores.

We also have some material on *Festuca ovina* L. (A. S. 20,527) from Skyway, Colo., with yellow spores  $29\text{--}38.6 \times 8.5\text{--}12.1 \mu$  borne on dead brown leaves on living plants. This fungus also is *St. simplicior* and similar to another collection on *F. ovina* var. *brachyphylla* from Skyway (10). A collection on *Sporobolus airoides* from Pullman, Wash. (FIG. 1J), was withheld from a monographic discussion of *Ascochyta* by myself and A. G. Johnson (11) because the spores had the same general shape as those of *St. simplicior* although they were very much smaller. It probably is a species of *Ascochyta* and resembles *A. boutelouae* Fairman which is the same as *Diplodina graminea*. A combination placing the last mentioned in *Ascochyta* was made elsewhere (11).

*Ascochyta stipae* Gonzalez-Fragoso (3) has ovoid-oblong spores  $7\text{--}8 \times 2.0\text{--}3.5 \mu$ . It is, therefore, entirely distinct from *St. simplicior*. *Ascochyella stipina* Petrak (4) also has much smaller spores than *St. simplicior*,  $7\text{--}13 \times 3\text{--}5 \mu$ .

SEPTORIA TRITICI Rob. in Desm. was found on dead leaves and sheaths of rye plants (*Secale cereale* L.) collected by B. F. Dana in acid soil at Shelton, Wash., June 23, 1919 (C. S. 679). The plants were also affected with *Ophiobolus graminis* Sacc. and *Rhizoctonia solani* Kuehn, both attacking the base of the culm (1). The *Septoria* has typical microspores and straight or curved 3-septate macrospores  $45\text{--}70 \times 1.4\text{--}1.7 \mu$ . The fungus was unquestionably *S. tritici* even though the host is certainly unusual. We have no record of this species on rye in this country. The host was growing in a humid region in acid, infertile soil and was attacked by rootrot and footrot organisms. This probably accounts for the presence of this fungus on the necrotic rye. *S. secalis* and *S. nodorum* have much wider-appearing spores than *S. tritici*.

SEPTORIA AVENAE Frank caused a tawny spot on the leaves of *Calamagrostis canadensis* L. at Baker Hole near West Yellowstone, Mont., collected Aug. 13, 1948 (A. S. 20,107), by Fischer, Meiners, and myself. The hyaline spores with somewhat granular contents were tapered towards the apex and definitely blunted at

the base (FIG. 1E). The spores were only 1-septate,  $21-28 \times 3.8-4.8 \mu$ . They are, therefore, shorter and broader than *S. avenae* on oats. The pycnidia are comparable, however, light golden brown,  $90-130 \mu$  diam., borne in typical lesions. This fungus could, of course, be classed as a 1-septate phase of *Stagonospora arenaria* Sacc., but this fungus appears to be less frequently encountered on the Agrostideae than on the Hordeae of the Gramineae. *S. avenae* was seen on *Agrostis exarata* Trin. from northern Idaho.

SEPTORIA ARUNDINACEA Sacc. was found on dead leaves of *Deschampsia caespitosa* (L.) Beauv. on the shore of Priest Lake, Ida., by the writer and Mary Sprague (A. S. 20,542). The spores in this material are very large,  $60-92 \times 4.8-6.0 \mu$ , slightly tinted, with small oil drops. They were bacillar, somewhat squared at the ends, slightly to scarcely constricted at the 3-6 septa, and somewhat brittle. The pycnidia were large, sometimes multilocular with 1 to 3 ostioles, strongly erumpent. This fungus is closer to *Septoria* than to either *Phaeoseptoria* or *Hendersonia*. This material is the first which I have seen from western America, although it probably is common on coarse marsh grasses in the eastern United States.

I doubt if the fungus is actively parasitic on *Deschampsia caespitosa*, a host resistant to most fungi.

ASCOCHYTA BRACHYPODII (Sydow) Sprague and A. G. Johnson (11) was found on *Andropogon scoparius* Michx. near Flagstaff, Ariz., by Sprague, Fischer, and Meiners (A. S. 20,543), on overwintered leaves. The spores had pointed ends as in North Dakota material on *Andropogon*. The collection represents an extension of range and host for the fungus.

SCAPHIDIUM BOUTELOUAE Clements was collected on *Bouteloua curtipendula* (Michx.) Torr. by W. G. Solheim and Ragnhild Solheim from the Santa Catalina Mts., Ariz. (Rocky Mtn. Herb. 2447) and Silver City, N. Mex. (Rocky Mtn. Herb. 2390). The fungus produces linear brown or gray lesions with brown borders, or in later saprophytic activity the entire lesion may be gray or dull black and with indefinite borders. In collection 2447, the brown-bordered lesion is surrounded by green tissue and is evidently the result of parasitic invasion of the living leaf. These lesions were  $1-4 \times 0.4-1$  mm. diam. with fawn-colored centers.

The pycnidia were black, flattened globose to somewhat elongated, breaking open at the apex and 60–100  $\mu$  diam. The spores were curved, fusiform-obclavate (FIG. 1F), yellow, 19–30  $\times$  2.8–5.3  $\mu$ . The type of this is not available for study, and I based my determination on the description.

PHLEOSPORA MUHLENBERGIAE Sprague and Solheim on *Muhlenbergia arizonica* Vasey was recently described (5). An illustration of the pycnospores is included at this time (FIG. 1G).

PHYLLOSTICTA OWENSII Sprague was originally found on *Dactylis glomerata* L. along the seacoast in Oregon. A. C. Goheen collected comparable material in a bog near Long Beach, Wash., July 19, 1949 (A. S. 20,510), on a very different host, *Panicum capillare* var. *occidentale* Rydb. The obscure lesions on the basal and sub-basal leaves are gray to brown. The pycnidia are small and black, containing bacillar-shaped spores, 3–4  $\times$  1.0–1.4  $\mu$ , which are exuded in agglutinated masses. This material is distinct from *P. panici* E. Young and *P. sorghina* Sacc.

SPERMOSPORA SUBULATA (Sprague) Sprague is associated with a light buff, elliptical red-bordered spot on *Trisetum spicatum* (L.) Richt., collected by G. W. Fischer in high country at Logan Pass, Glacier Nat'l Park, Mont. (A. S. 20,501). This is a new host-genus for the fungus. Fischer also collected the same fungus on *Bromus vulgaris* (Hook.) Shear in a devil's club thicket at Avalanche Lake in Glacier Nat'l Park, Mont. (A. S. 20,503). The lesions were gray, blasted areas on living leaves. A fungus which occurs on *Stipa lettermanii* Vasey near Cameron Lake, Waterton Nat'l Park, Alta., is also assigned to *S. subulata* (A. S. 20,506, legit. G. W. Fischer, 1949).

CERCOSPORA BOUTELOUAE Chupp and Greene was collected by the writer and Jack Meiners along Buckhorn Creek, Roosevelt Nat'l For., Colo., in Aug. 1948, on *Bouteloua gracilis* (H. B. K.) Lag., a new host. The spores were light brown, otherwise the same as the fungus described by Chupp and Greene. The narrow spots on living leaves have ashy centers, while those on dead leaves are usually uniformly dark because of the accumulation of conidio-phores. Part of this material was sent to Greene who forwarded it to Chupp for study. *C. boutelouae* is related to *C. apii*.

*OVULARIA PUSILLA* (Ung.) Sacc. and D. Sacc. causes a dark brown to sepia, elliptical spot on living leaves of *Alopecurus alpinus* J. E. Smith, near Hotel Many Glaciers, Glacier Nat'l Park, Mont. The material was collected Aug. 8, 1949, by G. W. Fischer (A. S. 20,505). Except for a collection of *Puccinia poae-sudeticae* (West.) Jorstd. from Colorado, this appears to be the only parasitic fungus reported on this unusual appearing alpine grass. *O. pusilla* was also collected on *Agrostis oregonensis* Vasey in Glacier Nat'l Park by Fischer. He also brought material of *Elymus glaucus* Buckl. from Kootenay Park, B. C., infected with *O. pusilla*. This seems to be the first report of the fungus from western Canada and the first report of it on the genus *Elymus*. The spots were elliptical in shape and fawn-colored.

*Ovularia pusilla* also causes a tan leaf blight on the filiform leaves of *Agrostis humilis* Vasey at Togwotee Pass in the Teton Nat'l For., Wyo. (Sprague, Fischer, and Meiners). This appears to be the first report of a parasitic fungus on this tufted alpine bent. *O. pusilla* is much more common on western grasses than is generally believed. The spores are so few in most water mounts that the fungus is frequently overlooked unless one is familiar with the symptoms or notes the small glistening tufts of conidiophores under a binocular ( $\times 45$ ).

*RHYNCHOSPORIUM ORTHOSPORUM* Caldwell was collected on *Agropyron subsecundum* by Alvin Law and Verne Comstock in Bountiful Canyon, Utah, July 13, 1949. The sub-hyaline spores were extremely variable in size and shape. A few had a faint tendency towards formation of obliquely hooked apices as in *R. secalis*. Some of the spores were straight and typical for *R. orthosporum*, averaging  $13-17 \times 3-4 \mu$ . Other spores were capsular shaped and so aberrant as to have little resemblance to the others. Some were distinctly swollen and others Indian-club-shaped. The capsular forms averaged about  $7 \times 4.2 \mu$ . These spores were borne in straw colored, elongate sheath spots with reddish brown borders. They resemble sharp eyespot (*Rhizoctonia solani* Kuehn) or straw-breaker eyespot (*Cercospora herpotrichoides* Fron) on wheat.

It is likely that the above-mentioned *Rhynchosporium* is not an undescribed species but an aberrant form of *R. orthosporum* modi-

fied by heat, following conditions that had been favorable to the fungus. We have seen unusual material of *Rhynchosporium* on *Elymus glaucus* Buckl., in Linn County, Oregon. This fungus, however, develops year after year in late winter in the same restricted area near Corvallis, Oregon. *R. orthosporum* has been collected on *Agropyron subsecundum* near Trude, Ida., but this material is not polymorphic as is the Utah collection. Typical material of *R. orthosporum* was also found on *A. subsecundum* var. *andinum* (Scribn. and Sm.) Hitchc. at an elevation of 11,316 feet on Fremont Pass, Colo. (A. S. 20,532). *R. secalis* was found on *A. subsecundum* at Pullman, Wash.

*RIHYNCHOSPORIUM SECALIS* (Oud.) J. J. Davis was collected on *Phalaris arundinacea* L. at Waterton Lakes, Alta., by G. W. Fischer. The spores are definitely intermediate between *R. orthosporum* and *R. secalis*, but it was assigned to the latter because the spores, or at least some of them, tended to be obliquely hooked at the apex. *R. secalis* was also collected on *Phalaris* near Leavenworth, Wash.

*STAGONOSPORA SUBSERIATA* (Desm.) Sacc. occurs in brown blotches on leaves of *Elymus mollis* Trin., collected by Austin Goheen on Neptune Beach (Puget Sound), Whatcom Co., Wash. (A. S. 20,539). This fungus was collected on the same host in Oregon (6) with spores  $20-33 \times 4.5-5 \mu$ . The material from Washington is worthy of note because it gives indications of parasitic activity. Our collections have pycnospores which are narrow for this species, but we believe that they represent the same organism. Goheen's specimen had sunken light brown pycnidia with the apex of each pycnidium protruding, ostiolate, spores yellow, guttulate, fusiform-cylindric, sometimes constricted at the septa,  $23-30 \times 4-5 \mu$ .

*SEPTORIA NODORUM* Berk. caused a prominent purplish brown glume blotch on heads of *Agropyron spicatum* (Pursh) Scribn. and Sm. growing in open woods north of Underwood, Wash. (legit. G. W. Fischer and Jack P. Meiners). The spores were 1-septate,  $15-18 \times 3.0-3.2 \mu$ . This is typical for the summer *Ascochyta* phase of *S. nodorum*. This is apparently the first report on *A. spicatum*. We also have material of *S. nodorum* on *Melica bulbosa* from Skyway, Colo. (A. S. 20,534). This collection has

circular to elongate, buff or fawn colored, sometimes vague spots, surrounded by brown tissue. The golden brown pycnidia have cylindrical hyaline or faintly greenish 1-septate spores,  $17-20 \times 2.4-3.4 \mu$ . This appears to be the same species as W. B. Cooke collected on *Melica californica* Scribn. on Mt. Shasta, Calif. (9). *S. nodorum* was collected on *Stipa viridula* Trin. at Gunnison, Colo. The spores were  $14-23 \times 2.2-2.8 \mu$ , mostly *Ascochyta*-like but somewhat more mature and 3-septate. It is difficult to segregate *S. nodorum* from immature material of *S. avenae*. On the basis of spore size, we must call these *S. nodorum*.

SCOLECOTRICHUM GRAMINIS Fekl. on *Glyceria leptostachya* Buckl., collected near Rochester, Wash. (A. S. 21,622), represents the first report of a parasitic fungus on this comparatively rare grass.

HELMINTHOSPORIUM DICTYOIDES Drechs. was collected on *Festuca pacifica* Piper near Colfax, Wash. (A. S. 20,518). *H. dictyoides* is not common in the west, even on *F. elatior*. The collection on *F. pacifica* represents the only known specimen of *H. dictyoides* on an annual species of fescue.

STAGONOSPORA AGROSTIDIS f. ANGUSTA Sprague was found mingled with rust pustules of *Puccinia scaber* (Ell. and Ev.) Barth. on leaves of *Stipa robusta* Scribn. near Stout, Colo. (A. S. 20,521). The spores were the same as in the type originally described on *Stipa viridula*.

SEPTORIA GLYCERICOLA Sprague was found in faded spots on *Glyceria grandis* S. Wats. near Lolo, Mont. (A. S. 20,524). The pycnidia were black, thick-walled, small ( $60-100 \mu$ ). The spores were also small,  $15-24 \times 1.6-1.9 \mu$ , 0-1-septate, filiform clavulate. This material compares with a collection on *G. grandis* from Bismarck, N. Dak. (8), which had summer spores,  $10-15 \times 1.3-1.6 \mu$ . These are believed to be summer phases of *S. glycericola*.

SEPTORIA ANDROPOGONIS J. J. Davis occurs on *Stipa robusta* Scribn. near Stout, Colo. (A. S. 20,528). The spores appear immature, 1-septate, blunt at the base, pointed at the apex,  $28-40 \times 2.6-3.5 \mu$ . We have found *S. andropogonis* f. *sporobolicola*, with spores  $40-73 \times 2.4-3.4 \mu$ , on *Stipa comata* Trin. and Rupr. at Mandan, N. Dak. (7). We have since found the same fungus on *Stipa viridula* in several states. The material on *Stipa robusta*

has shorter, proportionately stouter spores than *f. sporobolicola* but otherwise comparable. Some of the spores are ill-formed. Some have one cell empty, or the spores are distorted or twisted at the apex. The pycnidia are deep seated and occur in dull gray-brown lesions. The specimen on *S. robusta* possesses spores which are somewhat intermediate in size between *S. andropogonis* and *f. sporobolicola*. The entire series of collections available on coarse prairie grasses now indicates that *S. andropogonis* and its subdivisions are not segregated by sharp boundaries. Of course, the extremes, the extremely short *S. andropogonis* and the extremely long var. *sorghastri* Greene and Sprague, are readily distinguishable.

HELMINTHOSPORIUM TRITICI-REPENTIS Died. caused scattered small purple black spots on the leaves of a creeping form of *Agropyron spicatum* (Pursh) Scribn. and Sm. near Worley, Ida. (A. S. 20,544).

HELMINTHOSPORIUM STENACRUM Dreschl. was found on *Agrostis interrupta* L. near Ford, Idaho (A. S. 20,545). The dried brown basal leaves were covered with the fungus. The spores were  $65-105 \times 14-20 \mu$  diam. borne on simple conidiophores,  $60-135 \times 6.5-7.5 \mu$ . This collection is the first report of a fungus on this annual introduced bent grass from the west, and possibly from anywhere.

I am indebted to several collectors, but especially to G. W. Fischer and Jack P. Meiners for aid in gathering the material for study, and to Prof. Frank Potter, Emeritus Professor of Philosophy, W. S. C., for revising the Latin descriptions. I appreciate the assistance of the Editor, Alexander Smith, who has made editorial suggestions.

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## STUDIES IN THE LOWER CHYTRIDIALES. II. ENDO-OPERCULATION AND SEXU- ALITY IN THE GENUS DIPLO- PHLYCTIS<sup>1</sup>

R. H. HASKINS<sup>2</sup>

(WITH 10 FIGURES)

It is almost inevitable that in any extensive investigation (Haskins and Weston, 1950) of a group of organisms as diverse and as little known as are the lower Chytridiales, new and interesting things will be encountered. This was especially true in studies made on the *Diplophlyctis*-*Nephrochytrium* group of chytrids. On the basis of operculation, Sparrow (1943) has rather widely separated the inoperculate genus *Diplophlyctis* from the operculate genus *Nephrochytrium*. These two genera are alike in many ways, and hence confusion has arisen with the description of chytrids similar in more respects to *Diplophlyctis* than to *Nephrochytrium*, yet assigned to the latter genus because of the presence of "endo-opercula" (Karling, 1944). Investigations described in the present series of studies have revealed that there exist forms that may be either inoperculate or "endo-operculate."

Haskins (1939) mentioned a species of *Diplophlyctis* which was considerably coarser and larger than *Diplophlyctis intestina* (Schenk) Schroeter. This new species followed closely much of Karling's (1928, 1930, 1936) developmental description for *D.*

<sup>1</sup> This paper covers a portion of a dissertation presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Harvard University in 1948.

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Sincerest thanks are expressed to Professor Wm. H. Weston, of Harvard University, under whose kind and inspiring guidance these researches were carried out, to Dr. B. Boivin, Ottawa, for the Latin diagnosis and to The Edmund Niles Huyck Preserve Inc., Rensselaerville, New York, for research and collecting facilities during the summer of 1947.

*intestina* particularly in regard to variation in shape of zoösporangia, size of zoöspores, and morphology and germination of the resting structures. Further studies of this *Diplophlyctis* in connection with the present work have yielded additional information, for in addition to its larger size, the organism differs somewhat in thallus development, in the occasional presence of so-called "endo-opercula," and the presence of sexually-formed resting spores.

As for size, the spherical sporangia extend up to over  $170\ \mu$  in diameter, though the dimension in one direction may exceed this in irregular zoösporangia. The thallus appears very coarse when compared with the delicate thallus of *D. intestina* growing in *Nitella* internodes. The exit tubes, one or several, may be very short and broad, with reflexed rims, or may be long and narrow, extending up to  $150\ \mu$  from the main body of the zoösporangium.

In the development of the thallus there is a deviation from the sequence of events described by Karling (1930) for *D. intestina* and later confirmed by Sparrow (1936). According to their observations, the apophysis is laid down after the rudiment of the sporangium is formed. In *Nephrochytrium* the sporangium forms as a bud from the wall of the apophysis. In the present species, the sequence is as follows: at the end of a branch (usually a short lateral one) of a germ tube from the encysted zoospore, a tiny more or less dumb-bell-shaped swelling appears. The distal part of this swelling enlarges more rapidly than the proximal part (FIG. 1), and becomes the rudiment of the zoösporangium, while the proximal swelling enlarges to become the apophysis (FIGS. 2-4). The apophysis, then, appears at the same time as the sporangium. The connection or isthmus between the apophysis and the sporangium is always broad and has not been observed to be narrow as with *D. intestina* or with the species of *Nephrochytrium*.

The presence of so-called "endo-opercula" in this group has created considerable taxonomic uncertainty, similar to that to be described in a later paper of this series, for the *Rhizophlyctis*-etc. Group. *Nephrochytrium stellatum* Couch (Couch, 1938) dehisces "... always with a distinct cap," as does *Nephrochytrium aurantium* Whiffen (Whiffen, 1941). In *D. intestina* and *Diplophlyctis laevis* Sparrow the dehiscence is inoperculate. On the basis of the

presence of variously located "endo-opercula," Karling (1944) has placed *Nephrochytrium amazonensis* Karling in the genus *Nephrochytrium*, at the same time emphasizing its great similarity to the genus *Diplophlyctis*. Our species, in rapidly growing cultures, dehisces in a normal inoperculate manner and occasionally after extrusion of a gelatinous plug (FIG. 7), but in older stagnating cultures, after the deliquescence of the gelatinous tip of the exit-tube has occurred, the membrane between the protoplasmic content of the sporangium and the external medium thickens (FIG. 5). This membrane may thicken so as to form an endo-operculum-like structure (FIG. 8), which at subsequent dehiscence of the sporangium may merely rupture (FIG. 6) or may be forced out as a cap as described for *N. amazonensis* (Karling, 1944).

A study of the formation of the resting spores of the present strain yielded interesting and important information. The size of the resting structure (16-23  $\mu$ ) was found to be remarkably uniform, even when the organism was grown on media on which the vegetative thallus size varied greatly. This observation prompted a careful search for evidence of sexuality. This was soon found, but the sexuality was of a rather different type from that for which Sparrow (1936) had already presented evidence.

Though apparently normal resting spores were often found remote from other fungi on a piece of substrate, the majority were observed to be in groups of 2 to over 30 individuals. Examination of the smaller and more spread-out groups revealed the presence of small, thin-walled thalli which appeared to contain only the emptied cysts of zoöspores that had encysted in situ. From these cysts fine rhizoid-like germination tubes extended through the sporangial wall into the surrounding medium, where many of them were in open connection with the rhizoidal systems of fully developed resting spores (FIGS. 9-10).

When the group illustrated in figure 10 was first observed, the resting spore on the extreme left was hyaline, small, and obviously undeveloped with a thin, but faintly spiny wall. All of the cysts within what we may call the "male" thallus, were empty with the exception of the one which was connected with the above-mentioned rudimentary resting spore. The contents of this cyst in a few

hours had entered the connecting tube where the large refractive globule caused a local distension of the tube. This globule was observed to move slowly along the tube towards the resting structure and within about an hour had entered its apophysis. Observation had to be discontinued, but by the following day the globule was no longer in the apophysis, and the resting spore had become a brown, thick-walled, spiny resting structure. The material at this point was killed and fixed. Cytological studies of this phenomenon will be undertaken to provide supporting evidence that a sexual fusion has actually occurred.

The rhizoidal connections of the cysts within the thin-walled "male" thalli to the resting spores have been observed many times. Single normal resting spores have been found remote from all other thalli, and these have germinated normally, functioning as pro-sporangia to produce single sessile, thin-walled zoösporangia. This indicates that resting spores may also be formed asexually. Supporting the foregoing evidence for the presence of sexuality in this new species, are "male" thalli with contents in the cysts, but in connection with no other thalli—these have not been observed to develop further other than to produce the rhizoidal germination tubes (FIGS. 9-10). In addition to these isolated "male" thalli, are found isolated, small, hyaline, thin-walled, minutely-spiny, undeveloped resting spores, that also have never been observed to develop further presumably because rhizoidal outgrowths from the "male" thalli failed to reach them.

Because of the coarseness and larger size of the thalli, the manner of development of the thallus, the broad isthmus between the apophysis and the sporangium, the inoperculate but occasionally "endo-operculate" nature of the sporangium, and the type of sexuality, this organism is believed to be a species of *Diplophlyctis* other than *D. intestina* (Schenk) Schroeter and *D. laevis* Sparrow, and therefore is considered new. It is characterized by its relative coarseness and its hitherto undescribed type of sexuality, and is accordingly named *Diplophlyctis sexualis*.

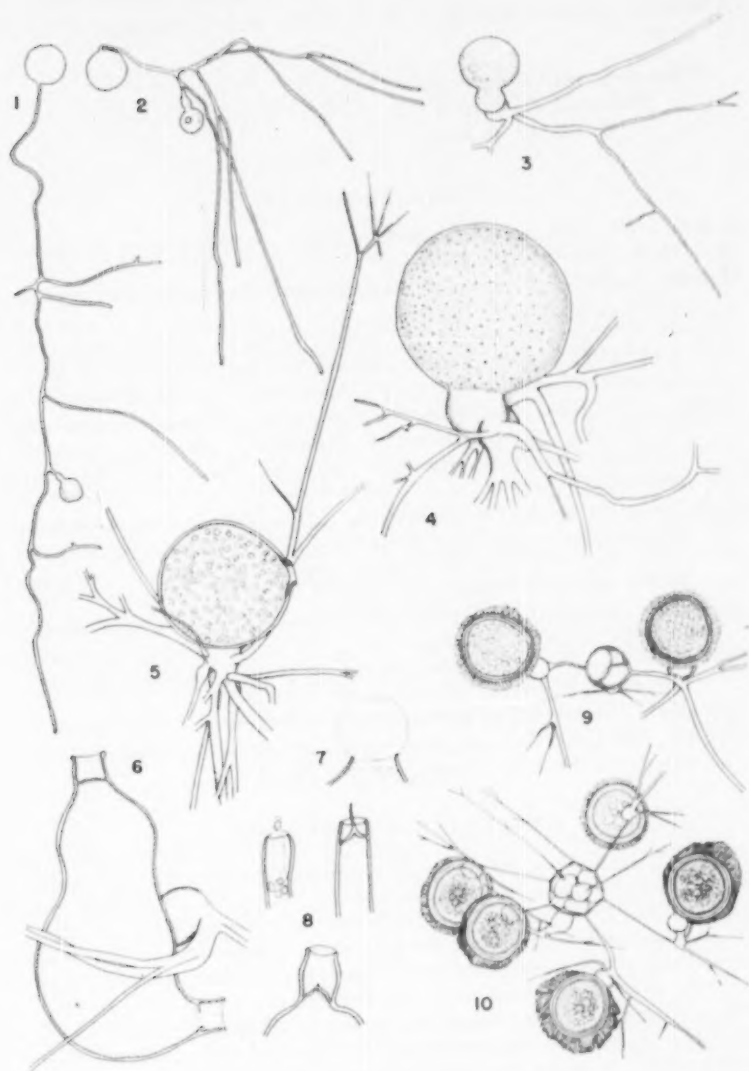
***Diplophlyctis sexualis* n. sp.**

Zoösporangii plerumque intramatricibus, laevibus, hyalinis, globosis vel variatim formati, 50-170  $\mu$ . Tubuli exeuntibus 1-5, brevibus rarius elonga-

tis, 10–150  $\mu$ , obturaculo gelatinoso, interdum cum endooperculis. Zoosporis globosis vel subglobosis, 5–6  $\mu$ , cum globulo uno refractivo, 2–4  $\mu$ . Vesiculo subsporangiali cum zoosporangio in isthmo amplo jugato, oriente uno atque eodem tempore quam sporangio. Rhizoidibus robustis. Sporis perdurantibus globosis, 16–23  $\mu$ , membrana crassa echinulataque, sexualiter vel asexualiter orientibus. Aplanogametibus masculis encystantibus in situ, i.e., in gametangio, copulatio per rhizoidales anastomosas. Sporis perdurantibus prosporangio vice in germinatione fungentibus ut surgat per poram exiguum zoosporangium extramaticale evanescens.

Asexual thallus monocentric, usually intramatrical, consisting of a sporangium or resting spore subtended by an apophysis from which arises a somewhat coarse, branched rhizoidal system. Sporangia hyaline, smooth, spherical, 50–170  $\mu$  in diameter, or variously shaped, walls not staining with zinc chloriodide. Exit tubes one to several, short and broad, or occasionally very long up to 150  $\mu$ . Dehiscence by deliquescence of tips of exit-tubes or by tip of tube softening to form an evanescent gelatinous plug beneath which an "endo-operculum" may or may not develop. Endo-opercula, when formed, shallow saucer-shaped, to cone-shaped with long spine. Zoospores spherical to sub-spherical, 5–6  $\mu$  in diameter each with single, large, prominent, highly refractive globule and a 30–40  $\mu$  flagellum, remaining temporarily motionless immediately after discharge, intermittently amoeboid. Apophysis spherical or variously shaped, joined to sporangium by a wide isthmus. Sporangium and apophysis originating at the same time at the end of an usually short branch of the germination tube from the encysted zoospore. Rhizoidal system usually arising from basal half of apophysis, stout, extensive, and much branched. Resting spores spherical, 16–23  $\mu$  in diameter; contents coarsely globular; wall thick, layered, dark brown, densely spiny, spines short and stout to long and hair-like; formed asexually or only after sexual fusion. Female thalli as for resting spores and remaining abortive unless fertilized. Male thalli consisting of a gametangium, apophysis and rudimentary sparsely-branched rhizoidal system. Gametangium hyaline, thin-walled containing 4 to many gametes. Gametes encysting in situ producing fine sparsely-branched rhizoid-like germination tubes which penetrate gametangium wall to anastomose with rhizoidal system of female thallus, through which anastomosis the content of the male cell passes to enter the female cell, which immediately develops into normal, brown, spiny-walled resting spore. Resting spores functioning as pro-sporangia in germination, each producing one sessile, thin-walled zoosporangium.

Saprophytic in natural habitat in decaying vegetable debris, readily developing on boiled leaves of maize, Cellophane, and lens paper



FIGS. 1-10.

submerged in water; collected in London, Ontario, Canada; Rensselaerville (Albany County), New York; and Cambridge, Mass., U. S. A.

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#### EXPLANATION OF FIGURES

FIGS. 1-2, Early stages in thallus development showing formation of rudiments of apophysis and enlarging sporangium. In earlier stages these are equal in size ( $\times 625$ ). FIGS. 3-5, Portions of maturing thalli ( $\times 225$ ). FIG. 6, Emptied zoösporangium showing walls ("endo-opercula") formed across bases of exit tubes under certain conditions ( $\times 188$ ). FIG. 7, Tip of exit tube showing gelatinous plug sometimes present in the tube ( $\times 188$ ). FIG. 8, Formation of operculum-like structures ("endo-opercula") within exit tubes, the tips of which have already deliquesced ( $\times 188$ ). FIG. 9, Two resting spores in connection with a male thallus ( $\times 500$ ). FIG. 10, Five mature resting spores in connection with cysts enclosed within a single male thallus ( $\times 500$ ).

(All figures drawn with aid of a Spencer Camera Lucida.)

## UREDINALES OF CONTINENTAL CHINA COLLECTED BY S. Y. CHEO. I<sup>1</sup>

GEORGE B. CUMMINS

(WITH 10 FIGURES)

The rusts reported in this paper were collected in cooperation between the Farlow Herbarium of Harvard University and the University of Nanking. All specimens are deposited in the Arthur Herbarium, Purdue University, and the Farlow Herbarium. Species preceded by an asterisk have not been recorded previously from continental China, insofar as I have been able to ascertain.

In general a conservative attitude has been adopted with respect to identifications; consequently only a few species have been described as new. This policy was followed because of the meager amount of Chinese material available for comparative study and because of uncertainty, in some cases, concerning the identity of the host material of Cheo's collections. With respect to both specimens and literature I have had the valuable assistance of Dr. Lee Ling of the United Nations Food and Agricultural Organizations as well as access to the rust specimens in the Chinese National Herbarium, now deposited in the Mycological Collections of the United States Department of Agriculture.

*PUCCINIASTRUM AGRIMONIAE* (Diet.) Tranz. On *Agrimonia* sp.: KWANGSI: Yung Hsien, Aug. 1933, (2588); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (384).

*PUCCINIASTRUM POTENTILLAE* Kom. On *Fragaria* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1155); KIANGSI: Sin Tsz Hsien, Sept. 1932, (1046).

<sup>1</sup> Cooperative investigations between the Purdue University Agricultural Experiment Station and the Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture. Journal Paper Number 450, of the Purdue University Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology. The first paper, based on the Cheo collections and covering the species of *Puccinia* on the Lauraceae, was published in Bull. Torrey Bot. Club 76: 31-38. 1949.

Only uredia are present but they agree well with those of *P. potentillae*, a rust previously recorded on *Potentilla fragarioides*. The specimen consists of trifoliate leaves having the appearance of *Fragaria*, but it is possible that the host is a species of *Potentilla*.

\*PUCCINIASTRUM HYDRANGEAE-PETIOLARIDIS Hirats. f. On *Hydrangea* sp.: KWEICHOW: Chiang K'ou Hsien, Nov. 1931, (872).

PUCCINIASTRUM CORIARIAE Diet. On *Coriaria sinica* Miq.: KWEICHOW: Tsunyi Hsien, Aug. 1931, (284). On *Coriaria* sp.: KWANGSI: Ling Yuin Hsien, Mar. 1933, (1730).

PUCCINIASTRUM TILIAE Miyabe. On *Tilia tuan* Szysz: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (521).

PUCCINIASTRUM CASTANEAEE Diet. On *Castanea* sp.: ANHWEI: Ch'ing Yung Hsien, Oct. 1932, (1385); KWEICHOW: Tsunyi Hsien, Aug. 1931, (273).

\*MELAMPSORIDIUM CARPINI (Fuckel) Diet. On *Carpinus* sp.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (606).

MELAMPSORA KUSANOI Diet. On *Hypericum patulum* Thunb.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (487), Tsunyi Hsien, July 1931, (150). On *Hypericum* sp.: KWANGSI: Ling Yuin Hsien, Mar., Apr. 1933, (1607, 1609, 1664, 1956).

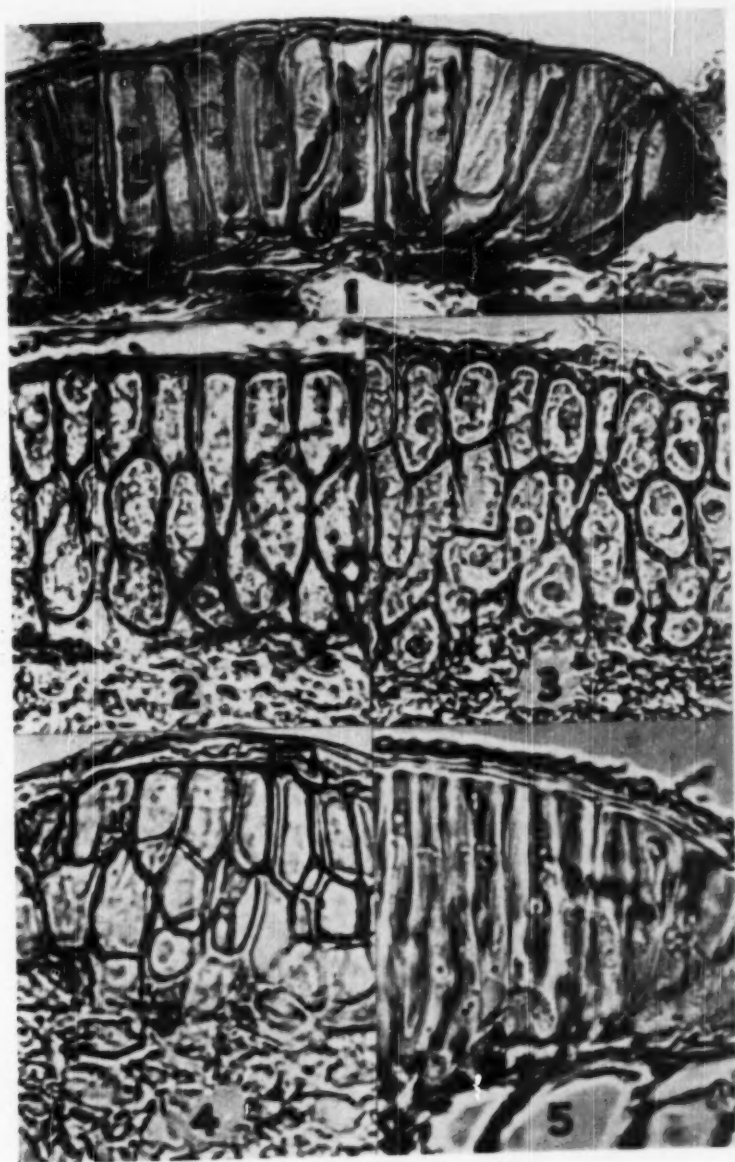
MELAMPSORA EUPHORBIAE-DULCIS Oth. On *Euphorbia chrysocoma glaucophylla* Levl.: KWEICHOW: Chiang K'ou Hsien, Oct. 1931, (656). On *Euphorbia pekinensis* Rupr.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (1022). On *Euphorbia* sp.: ANHWEI: Ch'ing Yang Hsien, Nov. 1932, (1501).

MELAMPSORA EUPHORBIAE (Schub.) Cast. On *Euphorbia* sp.: KWANGSI: Ling Yuin Hsien, June 1933, (2216), Lo Ch'en, Oct. 1933, (2871).

In Cheo's collections telia of *M. euphorbiae-dulcis* are present but only uredia of *M. euphorbiae*. The latter determination, consequently, is open to question.

\*MELAMPSORA YEZOENSIS Miyabe & Matsumoto. On *Salix longiflora* Anders.: KWEICHOW: Tsunyi Hsien, Aug. 1931, (263), Chiang K'ou Hsien, Sept. 1931, (370a).

No material of *M. yezoensis* has been available for comparison but telia are epiphyllous and subcuticular, with teliospores measuring  $7-12 \times 23-35 \mu$  and with the apical wall thin. The uredio-



FIGS. 1-5. Telia of *Melampsora* and *Phakopsora*.

spores have walls  $3-6\ \mu$  in thickness against  $3-10\ \mu$ , as given in the description of the species. The thickness of the urediospore wall separates this rust from *M. ribesii-viminalis* Kleb. although the telia are apparently similar. The latter species develops aecia on *Ribes* while the aecial host of *M. yezoensis* is *Corydalis*.

**\**Melampsora aleuritidis* sp. nov.**

Spermagoniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, sparsis vel aggregatis, plus minusve rotundatis,  $0.2-0.5$  mm. diam., flavidis, pulverulentis; paraphysibus plerumque capitatis,  $12-19 \times 40-55\ \mu$ , membrana hyalina,  $1.5-2\ \mu$  crassa; urediosporis late ellipsoideis vel obovatis,  $14-18 \times 18-24\ \mu$ ; membrana hyalina,  $2-2.5\ \mu$  crassa, moderate echinulata; poris germ. obscuris. Teliis (FIG. 1) hypophyllis, subepidermalibus, denseque aggregatis vel confluentibus, rotundatis,  $0.1-0.4$  mm. diam., aureo- vel castaneo-brunneis; teliosporis oblongis vel prismaticis,  $8-13 \times 31-43\ \mu$ ; membranibus ubique  $1-1.5\ \mu$  crassis, hyalinis vel pallide brunneis; statim germ.

On *Aleurites* sp.: KWEICHOW: Fan Ching Shan, Chiang K'ou Hsien, Oct. 19, 1931, S. Y. Cheo 730 (type!).

This is the first species of *Melampsora* reported as parasitizing *Aleurites* and is interesting, in addition, because the teliospores germinate without a period of dormancy.

MELAMPSORA LARICI-EPILEA Kleb.? On *Salix* sp.: ANHWEI: Ch'ing Yang Hsien, Nov. 1923, (1433, 1489, 1500, 1518, 1589); KWEICHOW: Tsunyi Hsien, July 1931, (56), Chiang K'ou Hsien, Sept. 1931, (370).

Most of these collections have both uredia and telia, the latter (FIG. 5) subcuticular with long narrow spores ( $4-9 \times 40-55\ \mu$ ) whose apical wall is not thickened.

While *M. larici-epitea* was described as having subepidermal telia Matsumoto (Trans. Sapporo Nat. Hist. Soc. 6: 11. 1915) includes both subcuticular and subepidermal rusts. It is extremely doubtful if such a procedure is correct and, consequently, none of Cheo's collection having telia (all except No. 370) probably represents this species. On the other hand agreement is poor with named subcuticular species because of the long, narrow, non-thickened teliospores.

Without adequate Asiatic *Salix* rusts for comparison the collections are arbitrarily assigned to *M. larici-epitea*, a rust recorded as occurring in China on three species of *Salix*.

MELAMPSORA COLEOSPORIOIDES Diet. On *Salix mesneyi* Hance: KIANGSI: Hsing Tsu Hsien, Sept. 1932, (1009).

\*CHNOOPSORA ITOANA Hirats. f. On *Oxalis* sp.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (414).

**\*Phakopsora malloti** sp. nov.

Spermogoniis et aeciis ignotis. Urediiis hypophyllis, subepidermalibus, minutis, 0.1-0.2 mm. diam., brunneis; peridiis hemisphaericis cum paraphysibus apicalis praeditis, paraphysibus incurvatis, 13-18  $\mu$  latis et usque ad 40  $\mu$  longis, membrana 1  $\mu$ , ad apicem 3-5  $\mu$  crassa; urediosporis subglobosis vel obovatis vel ellipsoideis, 16-20 (-22)  $\times$  (21-) 24-32 (-35)  $\mu$ ; membrana 1.5  $\mu$  crassa, pallide flavida vel brunnea, minuteque echinulata; poris germ. obscuris sed 4 vel 5, equatorialibus. Teliis (FIG. 3) hypophyllis, subepidermalibus, sparsis vel aggregatis, atro-brunneis, rotundatis, 0.2-0.5 mm. diam. vel confluentibus, ex sporis 2-6 irregulariter superpositis compositis; teliosporis variabilis, cuboideis, oblongis, vel plus minusve fusoides, 8-13  $\times$  13-30  $\mu$ ; membrana aureo-brunnea, ubique 1-1.5  $\mu$  vel ad apicem usque ad 2  $\mu$  crassa.

On *Mallotus* sp.: KWEICHOW: Fan Ching Shan, Chiang K'ou Hsien, Oct. 26, 1931, S. Y. Cheo 786 (type!).

If one follows Hiratsuka's treatment of the Japanese species of *Phakopsora* (Bot. Mag. Tokyo 49: 781-788. 1935), *P. malloti* would belong in the section *Physopella*, in which the uredia have incurved paraphyses and often also a basal peridium. There is no presently named species which should cause confusion. Hiratsuka (Bot. Mag. Tokyo 49: 23. 1935) has described *Pucciniastrum malloti* on *M. japonicus* from Formosa and based on uredia only. Through the courtesy of Dr. Ling I have seen some of this material, collected by Hashioka, and find that the peridium has apically free units and brownish spores. It may prove to be synonymous with the species named here.

PHAKOPSORA FORMOSANA Syd. On *Glochidion* sp.: KWANGSI: Ling Yuin Hsien, May 1933, (2094).

Only the aecial stage is present and it appears to be identical with *Aecidium innatum* Syd. & Butl. *P. innata* Sawada is doubtless synonymous.

\*PHAKOPSORA FICI-ERECTI Ito & Otani (FIG. 2)., On *Ficus* sp.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (961). On *Morus alba* L.: KIANGSI: Sin Tsz Hsien, Sept. 1932, (1050).

Recently, Tai (Farlowia 3: 98. 1947) described *P. hengshanensis*.

sis on *Ficus martini* and *F. heteromorpha* from Hunan Province. This species has not been available for study but, from the description, it could well be synonymous with *P. fici-erecti*. I have seen a rust on *Ficus beecheyana* from Formosa which appears to be the same as Cheo's collections. It has been determined by Sawada as *P. nishidana* but Hiratsuka (Mem. Tottori Agric. Coll. 7: 12. 1943; 7: 208. 1944) assigns the Formosan material to *P. fici-erecti* while recognizing *P. nishidana* as a separate species occurring on *F. carica*. He cites, as hosts of *P. fici-erecti*, *F. beecheyana*, *F. erecta*, *F. erecta* var. *sieboldii*, *F. formosana*, and *F. vasculosa*. Neither Tai nor Hiratsuka cites *Morus alba* as a host. A careful study, with adequate material, of these species would be desirable and should take into account the rust usually passing as *Cerotelium fici* (Butl.) Arth.

**\*Phakopsora cheoana sp. nov.**

Spermogoniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, brunneis, usque ad  $120\ \mu$  diam., paraphysibus periphericis, incurvatis, plus minusve capitatis,  $8-12 \times 30-45\ \mu$ ; membrana in partim ventralis  $1\ \mu$ , in partim dorsalis et apicalis  $3-5\ \mu$  crassa. Urediosporis late ellipsoideis,  $15-18 \times 22-26\ \mu$ ; membrana  $1-1.5\ \mu$  crassa, minuteque echinulata, flavida vel hyalina; poris germ. obscuris. Teliis (FIG. 4) hypophyllis, subepidermalibus, sparsis vel dense aggregatis, atro-brunneis, rotundatis, usque ad  $200\ \mu$  diam. vel plus minusve confluentibus, ex sporis 2 vel 3 superpositis compositis; teliosporis oblongis vel plus minusve cubicis,  $6-10 \times 10-23\ \mu$ ; membrana aureis vel pallide brunneis, ubique  $1.5\ \mu$  crassa.

On *Cedrela sinensis* Juss.: KWEICHOW: Fan Ching Shan, Chiang K'ou Hsien, Oct. 27, 1931, S. Y. Cheo 789 (type!).

There has been no species of *Phakopsora* described as occurring on the family Meliaceae. Since the uredia are old in this material and few spores are present it is possible that the description of the urediospores is not accurate.

\*PHAKOPSORA MEIBOMIAE Arth. On *Desmodium racemosum* (Thunb.) DC.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (575).

PHAKOPSORA PACHYRHIZAE Syd. On *Glycine sojae* Benth.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1224); KIANGSI: Sin Tsz Hsien, Sept. 1932, (1084); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (356). On *Glycine* sp.: ANHWEI: Ch'ing Yang Hsien,

Oct. 1932, (1231). KWANGSI: San Kiang Hsien, Sept. 1933, (2697). On *Pueraria thunbergiana* Benth.: KIANGSI: Sin Tsz Hsien, Sept. 1932, (1056); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (368). On *Shuteria* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (2763).

The rust on *Shuteria* is present as uredia and thus is only tentatively referred to this species.

\*PHAKOPSORA MELIOSMAE Kusano. On *Meliosma oldhami* Miq.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1274). On *Meliosma simplicifolia* Roxb.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1250). On *Meliosma* sp.: KWEICHOW: Fan Ching Shan, Oct. 1931, (794).

Thirumalachar and Kern (*Mycologia* 41: 288. 1949) have transferred this species to *Angiopsora* since they consider that the spores are catenulately produced.

PHAKOPSORA ZIZYPHI-VULGARIS Diet. On *Zizyphus jujuba* Mill. var. *inermis* (Bge.) Rehd.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (497). On *Zizyphus sativus* Gaertn.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1347). On *Zizyphus* sp.: KWANGSI: Lo Ch'en, Oct. 1933, (2875).

PHAKOPSORA AMPELOPSIDIS Diet. & Syd. On *Ampelopsis* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1363). On *Vitis pentagona* Diels & Gigl.: KWEICHOW: Sze Hsien, Aug. 1931, (344), Chiang K'ou Hsien, Sept. 1931, (390, 506). On *Vitis* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1169, 1179, 1380); KWANGSI: Yung Hsien, Oct. 1933, (2893).

This rust has been recorded on *Ampelopsis japonica*, *Vitis betulifolia*, and *V. vinifera* in China. Both uredia and telia are present on most of the above collections.

In 1935, Hiratsuka (*Bot. Mag. Tokyo* 49: 857) considered the common grape rust to be the same as the *Phakopsora* on *Ampelopsis*, thus reducing to synonymy *Uredo vitis* Thuem. and telial names based on it. This is undoubtedly correct since all specimens seen with telia, including American material, seem identical. Thirumalachar and Kern (*Mycologia* 41: 288. 1949) consider this species to be an *Angiopsora* and have, consequently, made the transfer.

*PHAKOPSORA PUNCTIFORMIS* (Barcl. & Diet.) Diet. On *Galium aparine* L.: KWEICHOW: Tsunyi Hsien, Aug. 1931, (199).

*PHAKOPSORA ARTEMISIAE* Hirats. f. On *Artemisia* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1145, 1159); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (584, 613). On *Aster* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1255); KIANGSI: Hsing Tzu Hsien, Sept. 1932, (1027). On *Chrysanthemum* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1332).

Another species, *Phakopsora compositarum* Miyake, occurs in China but is not represented in Cheo's collections. Both species are recognized by Hiratsuka (*l.c.*). *P. artemisiae* has mostly epiphyllous uredia, urediospores  $24-36\ \mu$  long, and teliospores  $19-33\ \mu$  long while in *P. compositarum* the uredia are hypophyllous, the urediospores  $24-28\ \mu$  long, but the teliospores  $24-60\ \mu$  in length. The latter species has not been available to me.

\**Phakopsora incompleta* (Syd.) comb. nov. (*Puccinia incompleta* Syd. Annal. Mycol. 10: 261. 1912). On *Andropogon* sp.: KWANGSI: Yung Hsien, Oct. 1933, (2904). On *Eulalia* sp.: KWANGSI: Ta Tseh Tsuen, Aug. 1933, (2311, 2420).

This rust, originally described from India on *Ischaemum* and subsequently reported for Formosa and the Philippines, apparently also occurs in Africa on *Andropogon* and possibly *Exothea*. In addition to the African specimens I have seen two Indian collections, including the type. While the uredia and urediospores are essentially alike in all specimens the only telia that I have seen are in Cheo's Nos. 2311 and 2904. Sydow reported seeing only two immature teliospores but gave no measurements nor did he mention pedicels.

Since, from specimens now available, it is apparent that the rust is not a species of *Puccinia*, the species is transferred to *Phakopsora* with the following description.

Uredia hypophyllous, scattered, minute, 0.1-0.2 mm. diam., with abundant peripheral, incurved, hyaline or pale golden paraphyses,  $8-13 \times 35-45\ \mu$ , the outer (convex) wall  $2-4\ \mu$  thick, the inner (concave) wall  $1-1.5\ \mu$  thick; urediospores obovoid, ellipsoid, or broadly ellipsoid,  $15-19 \times 19-26\ \mu$ , wall pale brownish to nearly hyaline, finely and closely echinulate,  $1-1.5\ \mu$  thick, pores probably scattered, obscure. Telia mostly hypophyllous, minute, oval or

ellipsoid, 0.2–0.5 mm. long, chestnut-brown, subepidermal and indehiscent, 1–3 spores in thickness; teliospores irregularly arranged, variable, more or less oblong,  $8-15 \times 18-26$  ( $-35$ )  $\mu$ ; wall golden or pale brownish, uniformly 1–1.5  $\mu$  thick, smooth.

*CRONARTIUM QUERCUM* Miyabe. On *Quercus* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (2789); KWEICHOW: Sze Nan Hsien, Aug. 1931, (351), Chiang K'ou Hsien, Sept. 1931, (609).

*COLEOSPORIUM CLEMATIDIS* Barcl. On *Clematis grata* Wall. var. *grandidentata* Rehd. & Wils.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (403). On *Clematis montana* Buch.-Ham.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (616). On *Clematis* sp.: ANHWEI: Ch'ing Yang Hsien, Nov. 1932, (1541); KIANGSI: Hsing Tzu Hsien, Nov. 1932, (924); KWANGSI: San Kiang Hsien, Sept. 1933, (2769, 2809, 2861); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (408, 395, 535), Tungjen Hsien, Dec. 1931, (891), Tsunyi Hsien, July 1931, (105, 161).

*COLEOSPORIUM EVODIAE* Diet. On *Evodia meliaefolia* Benth.: KWEICHOW: Sze Nan Hsien, Aug. 1931, (347), Tsunyi Hsien, July 1931, (46). On *Evodia officinalis* Dode: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1190). On *Evodia* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1286, 1382); KWANGSI: Ling Yui Hsien, Mar. 1933, (1644), Yung Hsien, Aug. 1933, (2305), Sze Nan Hsien, Aug. 1931, (347).

*COLEOSPORIUM XANTHOXYLI* Diet. & Syd. On *Zanthoxylum* sp.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (489).

**\**Coleosporium violae* sp. nov.**

Spermagoniis et acciis ignotis. Urediis hypophyllis, subepidermalibus, flavidis, plus minusve pulverulentis, 0.1–0.5 mm. diam.; urediosporis globoideis vel late ellipsoideis,  $13-18 \times 17-24$   $\mu$ ; membrana verrucosa, hyaline, 1.5–2  $\mu$  crassa; poris germ. obscuris. Teliis hypophyllis, sparsis, rotundatis, 0.1–0.4 mm. diam. vel confluentibus, ceraceis, pallide aureis; teliosporis cylindraceis, utrinque rotundatis,  $13-20 \times 43-56$   $\mu$ ; episporio hyalino, 1  $\mu$  crasso, ad apicem 8–15  $\mu$  et valde gelatinoso; statim germ. Basidiosporis non visis.

On *Viola limprichtiana* W. Becker: KWEICHOW: Ling Feng Yah, Tsunyi Hsien, Aug. 11, 1931, *S. Y. Cho* 289 (type!).

Species of *Coleosporium* have not been collected on Violaceae previously.

**\*Coleosporium cheoanum** sp. nov.

Spermogoniis et aeciis ignotis. Urediis hypophyllis, subepidermalibus, sparsis, flavidis, rotundatis, 0.1-0.4 mm. diam., pulverulentis, urediosporis late ellipsoideis vel globoideis,  $14-18 \times 16-21 \mu$ ; membrana verrucosa, hyaline,  $1.5-2 \mu$  crassa; poris germ. obscuris. Teliis hypophyllis, subepidermalibus, rotundatis, 0.1-0.5 mm. diam., ceraceis, aureis; teliosporis cylindraceutis, utrinque rotundatis,  $13-19 \times 32-52 \mu$ ; membrana hyalina,  $1 \mu$  crassa, ad apicem  $3-8 \mu$ , lamina gelatinosa inconspicua vel vix praedita; statim germ., basidiosporae  $10-14 \times 14-18 \mu$ .

On *Coleus* sp.: KWEICHOW: Fan Ching Shan, Chiang K'ou Hsien, Sept. 21, 1931, *S. Y. Cheo* 548 (type!).

*C. cheoanum* differs from other species which parasitize the Labiatae because of the small urediospores and teliospores. In addition the apical wall of the teliospores is unusually thin and shows little or no gelatinization.

**\*Coleosporium rubiicola** sp. nov.

Spermogoniis et aeciis ignotis. Urediis hypophyllis, sparsis, flavidis, rotundatis, 0.5-1.0 mm. diam., pulverulentis; urediosporis late ellipsoideis vel oblongo-ellipsoideis vel oblongis,  $13-20 \times 23-34$  (-38)  $\mu$ ; episporio hyalino, verrucoso,  $2 \mu$  crasso; poris germ. obscuris. Teliis hypophyllis, subepidermalibus, ceraceis, aureis, rotundatis, 0.3-1.0 mm. diam., sparsis; teliosporis cylindraceutis, utrinque rotundatis vel deorsum attenuatis,  $15-19 \times 100-125 \mu$ ; episporio hyalino,  $1 \mu$  crasso, ad apicem gelatinoso  $12-20 \mu$  crasso; statim germ.

On *Rubia cordifolia* L.: KWEICHOW: Fan Ching Shan, Tungjen Hsien, Nov. 6, 1931, *S. Y. Cheo* 810 (type!).

Of the species parasitizing Rubiaceae *C. rubiicola* has the longest urediospores and teliospores. The teliospores are among the longest described in *Coleosporium*. *C. knoxiae* Syd. has nearly as long teliospores,  $65-100 \mu$ , but the urediospores are smaller,  $15-20 \times 17-25 \mu$ . I have been unable to find a previous report of a *Coleosporium* on the genus *Rubia*.

COLEOSPORIUM PLECTRANTHI Barcl. On *Plectranthus racemosus* Hemsl.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (898). On *Plectranthus* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1299); KWANGSI: San Kiang Hsien, Sept. 1933, (2844).

COLEOSPORIUM PERILLAE Syd. On *Mosla chinensis* Maxim.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1180). On *Mosla* sp.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (928); KWEICHOW:

Chiang K'ou Hsien, Sept. 1931, (494), Tsunyi Hsien, July, Aug. 1931, (4, 191). On *Perilla ocymoides* L.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1111); KIANGSI: Hsing Tzu Hsien, Sept. 1932, (896). On *Perilla* sp.: KIANGSI: Sin Tsz Hsien, Sept. 1932, (1039); KWANGSI: Yung Hsien, Aug., Oct., 1933, (2526, 2887); KWEICHOW: Tsunyi Hsien, July 1931, (121), Sze Nan Hsien, Aug. 1931, (336).

COLEOSPORIUM PAEDERIAE Diet. On *Paederia* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (2806); KWEICHOW: Sungtao Hsien, Nov. 1931, (815).

COLEOSPORIUM CAMPANULAE Lév. On *Adenophora* sp.: KIANGSI: Sin Tze Hsien, Sept. 1932, (1044). On *Platycodon* sp.: KWEICHOW: Fan Ching Shan, Sept. 1931, (355). On *Lobelia pyramidalis* Wall.: KIANGSI: Sin Tsz Hsien, Sept. 1932, (1058). On *Lobelia* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (2728); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (457).

COLEOSPORIUM ASTERUM (Diet.) Syd. On *Aster* sp.: ANHWEI: Ch'ing Yang Hsien, Oct., Nov., 1932, (1252, 1441, 1542, 1552); KIANGSI: Hsing Tzu Hsien, Sept. 1932, (1029); KWANGSI: Ling Yuin Hsien, June 1933, (2290), San Kiang Hsien, Sept. 1933, (2821); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (549), Tsungi Hsien, July, Aug. 1931, (49, 322).

COLEOSPORIUM CARPESII Sacc. On *Carpesium* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1127); KIANGSI: Hsing Tzu Hsien, Sept. 1932, (900); KWANGSI: Ling Yuin Hsien, June 1933, (2211), Yung Hsien, Oct. 1933, (2894).

\*COLEOSPORIUM ELEPHANTOPODIS Thuem. On *Elephantopus* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (2860).

COLEOSPORIUM EUPATORII Arth. On *Eupatorium lindleyanum* DC.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1240). On *Eupatorium* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (2696); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (362, 397).

COLEOSPORIUM SAUSSUREAE Thuem. On *Saussurea* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1217); KWANGSI: San Kiang Hsien, Sept. 1933, (2778); KWEICHOW: Tsunyi Hsien, July, Aug. 1931, (29, 228).

\*COLEOSPORIUM sp. On *Siegesbeckia orientalis* L.: KWEICHOW: Tsunyi Hsien, July 1931, (34).

I find no record of a species *Coleosporium* parasitizing *Siegesbeckia*, a genus of the tribe Heliantheae. Only uredia are present.

*COLEOSPORIUM BLETIAE* Diet. On *Anthogonum* sp.: KWEICHOW: Tsunyi Hsien, Aug. 1931, (241).

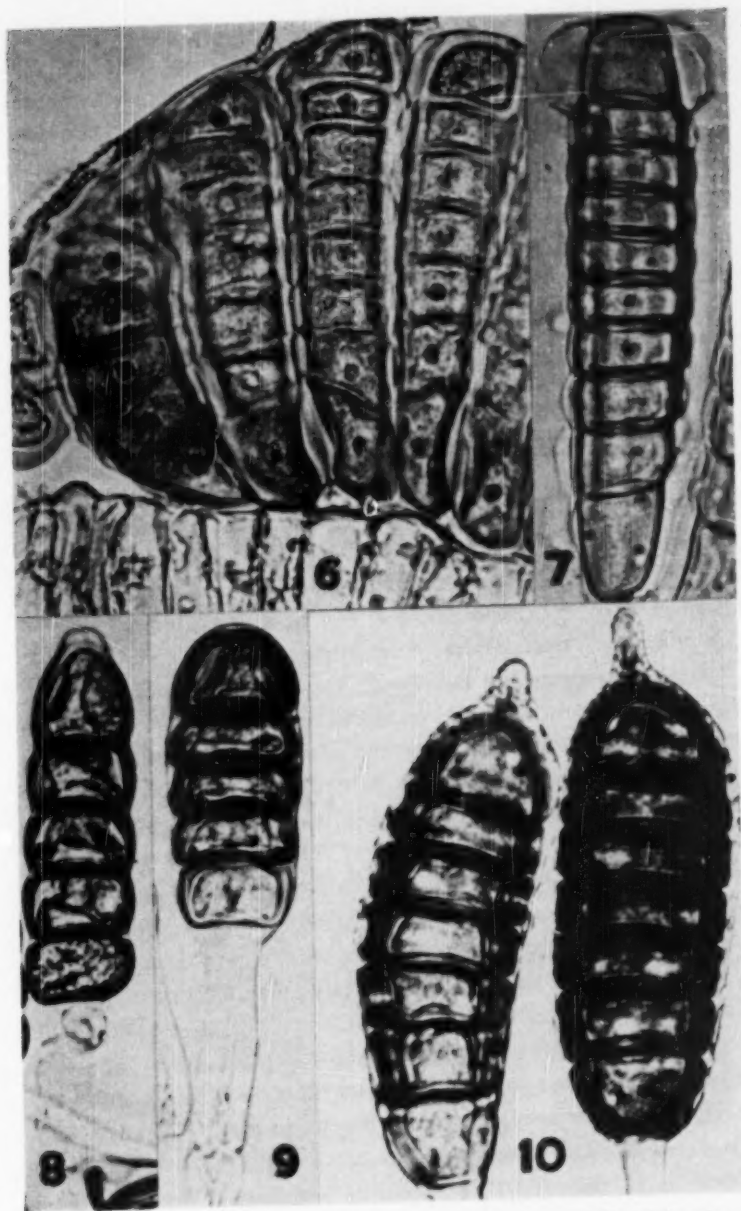
*PUCCINIOSTELE MANDSCHURICA* Diet. On *Astilbe chinensis* Franch. & Sav.: KWANGSI: San Kiang Hsien, Sept. 1933, (2727).

\**Pucciniostele hashiokai* (Hirats. f.) comb. nov. (*Cerotelium hashiokai* Hirats. f., Jour. Jap. Bot. 13: 248. 1937; *Pucciniostele ampelopsidis* Sawada, Formosan Agr. Rev. 38: 703. 1942) (FIGS. 6, 7). On *Ampelopsis* sp.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (514); KWANGSI: Yung Hsien, Oct. 1933, (2939).

Through the courtesy of Y. Hashioka I was able to examine a Formosan specimen collected on *Ampelopsis cantoniensis* Planch. at Rengechi, Prov. Taichu, Nov. 3, 1932, by Hashioka. The type is Hashioka No. 218, collected at Gojyo, but both were cited in Hiratsuka's original description. The type of *Pucciniostele ampelopsidis* was furnished, together with a translation of Sawada's description, by Dr. Lee Ling. Sawada's specimen was collected at Urai on the same host. It is probable that the host of Cheo's collections is also *A. cantoniensis* and certainly the rust is the same in all collections.

While Hiratsuka's description is sufficiently accurate it is obvious that the rust is not a species of *Cerotelium* since each cell of the teliospore has two or three equatorially placed germ pores. While more difficult to observe this is apparently also true of *Pucciniostele clarkiana* (Barcl.) Diet. and *P. mandschurica*. In *P. hashiokai* the outer wall of the spore is somewhat hygroscopic, especially over the germ pores where it swells considerably (FIG. 7). The spores do not adhere laterally and when mature give much the impression of a *Phragmidium* spore minus its pedicel.

The uredia are phakopsoroid in character with peripheral, incurved, dorsally thickened, septate paraphyses. With only uredia present it would be difficult accurately to distinguish this rust and *Phakopsora ampelopsidis* Diet. & Syd. While the two other species of *Pucciniostele* are described as lacking uredia I have found evidence, in Philippine specimens of *P. clarkiana*, that uredia occur in that species. Primary stages are unknown for *P. ampelopsidis*.



FIGS. 6-10. Telium of *Pucciniostele* and teliospores of *Pucciniostele* and *Phragmidium*.

\**RAVENELIA ATRIDES* Syd. On *Grewia parviflora* Bunge: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (964).

This rust has not been recorded from China although it occurs in the Philippines. Telia have been found only in Africa, however.

\**RAVENELIA INDIGOFEAE* Tranz. On *Indigofera* sp.: KWANGSI: Ling Yui Hsien, June 1933, (2273).

Only uredia are present, which makes the identification uncertain.

*RAVENELIA JAPONICA* Diet. & Syd. On *Albizzia* sp.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (929); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (475).

*PILEOLARIA KLUGKISTIANA* Diet. On *Rhus punjabensis* Stewart var. *nucipersica* (L.) Schneid.: KWEICHOW: Tsunyi Hsien, July 1931, (127). On *Rhus* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1114); KIANGSI: Hsing Tzu Hsien, Sept. 1932, (1031); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (509).

*PILEOLARIA SHIRAIANA* (Diet. & Syd.) Ito. On *Rhus* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1105); KIANGSI: Hsing Tzu Hsien, Sept. 1932, (1025); KWANGSI: Yung Hsien, Aug. 1933, (2459), San Kiang Hsien, Sept. 1933, (2695, 2815); KWEICHOW: Tsunyi Hsien, Aug. 1931, (260).

These two species of *Pileolaria*, both previously recorded from China, are readily separable on the basis of either urediospores or teliospores. In *P. klugkistiana* the teliospores are deep chestnut-brown but not opaque, and the sculpturing consists of variously anastomosing ridges or warts which results in an irregularly and rather indistinctly reticulate pattern. The teliospores of *P. shiraiana* while immature are smoky or almost olivaceous brown, but with maturity become so densely pigmented and opaque that the thickness of the wall cannot be observed. There is no indication of reticulation but the wall is uniformly verrucose with somewhat conical warts.

Neither the number nor the location of the germ pores of the urediospores is given by the Sydows (Monogr. Ured. 3: 145, 150. 1910) for either species. In *P. klugkistiana* the pores are four, as stated by Tai (Nanking Jour. 2: 8. 1932), and equatorial, while in *P. shiraiana* there are two indistinct pores adjacent to the hilum. The wall in both species is spirally adorned with

widely spaced ridges, homogeneous in structure in *P. klugkistiana* but beaded in *P. shiraiana*. Careful examination of the ridges of the latter species reveals that the ridges are actually continuous but with internal differentiation which, because of unequal refraction, gives a beaded appearance.

TRANZSCHELIA PRUNI-SPINOSAE (Pers.) Diet. On *Prunus persica* (L.) Stokes: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (904); KWANGSI: Ling Yuin Hsien, May 1933, (2102). On *Prunus* sp.: KWANGSI: Ling Yuin Hsien, June 1933, (2289).

\*KUEHNEOLA CALLICARPAE Syd. On *Callicarpa* sp.: KWANGSI: San Kiang Hsien, Sept. 1933, (2766).

GERWASIA CHINENSIS (Diet.) Hirats. f. On *Rubus amplifolius* Levl. & Van: KWEICHOW: Chiang K'ou Hsien, Oct. 1931, (777), Tungjen Hsien, Dec. 1931, (890). On *Rubus setchuensis* Wright: KWEICHOW: Tsunyi Hsien, July 1931, (35). On *Rubus* sp.: KWANGSI: Ling Yuin Hsien, Mar., Apr. 1933, (1610, 1858, 1889).

This appears to be a common Asiatic species. It remains a question how closely it may be related to *Gerwasia rubi* Racib., described from Java. No one has succeeded in obtaining Raciborski's type but recently Hiratsuka (Mem. Tottori Agric. Coll. 3: 26. 1943) has reduced the above name to synonymy with *G. rubi*, which may well be correct. In this publication he does not include *G. fasciculata* Arth. & Cum., although he had previously reduced it to synonymy under *G. chinensis*. Both species are superstomatal but *G. fasciculata* is paraphysate and doubtless a distinct species.

GERWASIA ROSAE Tai. On *Rosa roxburghii* Tratt.: KWEICHOW: Tsunyi Hsien, July 1931, (86). On *Rosa* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1384); KWANGSI: Ling Yuin Hsien, Apr. 1933, (1949).

Only uredia are present in these collections and since Tai (Farlowia 3: 103. 1947) described only telia the identification is uncertain. The uredia, however, correspond closely in structure to the telia described by Tai. The spores are produced at the apex of slender basal cells. The sori are at first superstomatal but later rupture the epidermis, and may attain a diameter of 100  $\mu$  or more. Incurved, unilaterally thickened paraphyses, borne in the same

manner as the teliospores, are abundant. The paraphyses are one-septate, a characteristic not mentioned by Tai.

If these collections are not related to *Gervasia* one would expect them to belong with an undescribed *Kuchneola*. There is, in the Philippines, a species of *Kuchneola*, thus far erroneously referred to *K. japonica*, which has uredinoid aecia whose spores are similar to the urediospores of Cheo's collections. Smaller, less abundant, non-septate, but otherwise similar paraphyses occur in these aecia. Telia are present in the three available specimens but no true uredia could be found. Presumably the species is an *opsis* type. Since *K. japonica* is microcyclic the Philippine rust cannot be identical; and since the Chinese specimens have uredia, smaller spores, and larger septate paraphyses they cannot be identified with the Philippine rust. A description of the uredia of the Chinese rust follows:

Uredia hypophyllous, usually closely grouped on brownish spots obvious on the upper side of the leaf, or the distribution more diffuse, minute, usually less than  $100\ \mu$ , subepidermal or merely superstomatal when first formed, yellowish in dry condition; paraphyses abundant, incurved, hyaline or nearly so, 1-septate near the base, borne on basal cells with the urediospores, the wall  $3\text{--}5\ \mu$  thick on the convex side,  $1\ \mu$  on the concave side; urediospores ovoid, obovoid, or ellipsoid, usually with three slightly protruding angles in the equatorial zone,  $16\text{--}19\text{--}(21) \times 21\text{--}26\text{--}(29)\ \mu$ ; wall  $1\text{--}1.5\ \mu$  thick, hyaline or pale yellowish, finely and closely echinulate, the pores obscure but probably 3, equatorial and located in the angles.

HAMASPORA ACUTISSIMA Syd. On *Rubus setchuensis* Wright: KWEICHOW: Sze Nan Hsien, Aug. 1931, (350). On *Rubus* sp.: KIANGSI: Hsing Tzu Hsien, Sept. 1932, (957); KWANGSI: Ling Yui Hsien, Apr. 1933, (1841, 1947); KWEICHOW: Tsunyi Hsien, Aug. 1931, (145).

HAMASPORA BENGUETENSIS Syd. On *Rubus* sp.: ANHWEI: Ch'ing Yang Hsien, Nov. 1932, (1135); KWEICHOW: Tsunyi Hsien, Aug. 1931, (220).

Telia are not present in either collection. Since *H. benguensis* and *H. rubi-sieboldii* (Kawagoe) Diet. differ mainly in teliospore morphology, identity of these collections is uncertain. *H. benguensis* has been reported from China but *H. rubi-sieboldii* has not.

\*HAMASPORA HASHIOKAI Hirats. f. On *Rubus* sp.: KWANGSI: Ling Yuin Hsien, Apr. 1933, (1948).

The most distinctive feature of this species is provided by the urediospores, which have a thickened apical wall. Reports of the occurrence of *H. longissima* (Thuem.) Koern. in China are probably erroneous and in part, at least, referable to *H. hashiokai*. There is, in the Arthur Herbarium, a specimen on *Rubus* sp. from Sikiang (*Teng 3294a*) which is certainly *H. hashiokai* and in Tai's "List" (*Sci. Rept. Natl. Tsing Hua Univ. Ser. B. 2: 297-418. 1937*) *H. longissima* is recorded on *Rubus lambertianus*, which is the host of *H. hashiokai*.

HAMASPORA SINICA Tai & Cheo. On *Rubus* sp.: KWANGSI: Ling Yuin Hsien, Mar. 1933, (1762) (issued as *H. acutissima* Syd. in *Reliq. Farlowianae* No. 730), Apr. 1933, (1822, 1825).

A portion of the type has been available for comparison. There is considerable chance of confusing *H. sinica*, *H. taiwaniana* Hirats. f. & Hashioka, and *H. acutissima* since the number of septations and the degree of apical thickening of the teliospores vary considerably. In addition, uredia have not been described for *H. sinica* and *H. taiwaniana*.

PHRAGMIDIUM POTENTILLAE (Pers.) Karst. On *Potentilla* sp.: Ling Yuin Hsien, Mar. 1933, (1729).

PHRAGMIDIUM GRISEUM Diet. On *Rubus* sp.: ANHWEI: Ch'ing Yang Hsien, Oct., Nov. 1932, (1123, 1466).

The teliospores of this species (FIG. 8) are usually narrower than those of *P. rubi-thunbergii* (FIG. 9) and the apex abruptly narrowed and umbonate, with the wall of the umbonate portion subhyaline and thickened. Both species belong in the section *Phragmotelium*, which is frequently treated as a distinct genus. I am following Hiratsuka's concept as presented in his treatment of the Japanese species of *Phragmidium* (*Jour. Jap. Bot. 7: 227-299. 1935*).

Observations of Cheo's collections referable to the section *Phragmotelium* reveal a character which appears not to have been recognized previously. There is a septum present which separates the pedicel proper from a basal portion which is of approximately the same diameter but thinner-walled. Normally the basal portion

does not remain attached to the pedicel but can be seen readily in carefully prepared mounts (FIG. 9). An examination of other available species indicates that the septate pedicel may be characteristic of all species of the section.

*PHRAGMIDIUM RUBI-THUNBERGII* Kusano (FIG. 9). On *Rubus thunbergii* Sieb. & Zucc.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1261). On *Rubus* sp.: KWEICHOW: Tsunyi Hsien, July, Aug. 1931, (23, 32, 206).

\**PHRAGMIDIUM NAMBUANUM* Diet. On *Rubus* sp.: KWEICHOW: Chiang K'ou Hsien, Oct. 1931, (658).

*PHRAGMIDIUM MUCRONATUM* (Fr.) Schl. On *Rosa* sp.: KWEICHOW: Chiang K'ou Hsien, Oct. 1931, (739).

*PHRAGMIDIUM ROSAE-DAHURICAE* Miura. On *Rosa* sp.: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1381, 1392).

No material of this species has been available for comparison but Cheo's specimens agree well with Miura's description. The teliospores (FIG. 10) are chestnut-brown but not opaque and the telia, likewise, are not blackish brown as in most species on roses.

*LEUCOTELIUM PRUNI-PERSICAE* (Hori) Tranz. On *Prunus persica* (L.) Stokes: ANHWEI: Ch'ing Yang Hsien, Oct. 1932, (1112); KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (463). On *Prunus* sp.: KWEICHOW: Chiang K'ou Hsien, Sept. 1931, (371).

As pointed out by Butler and Bisby (Fungi of India, p. 72. 1931), Tai (Nanking Jour. 2: 172. 1932), and Tranzschel (Conspectus Ured. URSS, p. 238. 1939) the urediospores described for *Puccinia pruni-persicae* by Hori (Phytopath. 2: 144. 1912) are actually those of *Tranzschelia pruni-spinosae* and have no connection with the telia. The uredia of the species are pale brownish and have capitate paraphyses somewhat as in *Tranzschelia*, but the urediospores are pale brownish or yellowish, the wall is uniformly echinulate, and uniformly  $1.5\ \mu$  in thickness.

\**MARAVALIA ACHROA* (Syd.) Arth. & Cum. On *Dalbergia* sp.: KWEICHOW: Sze Nan Hsien, Aug. 1931, (327).

THE ARTHUR HERBARIUM,  
PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION,  
LAFAYETTE, INDIANA

## DESCRIPTION OF FIGURES

FIG. 1, telium of *Melampsora aleuritidis* Cum. on *Aleuritis* sp. (from type); 2, telium of *Phakopsora fici-erecti* Ito & Otani on *Ficus* sp. (from Cheo 961); 3, telium of *Phakopsora malloti* Cum. on *Mallotus* sp. (from type); 4, telium of *Phakopsora cheoana* Cum. on *Cedrela sinensis* (from type); 5, telium of *Melampsora larici-epitea* Kleb.? on *Salix* sp. (from Cheo 1518). All photos are from unstained free-hand sections.  $\times 800$ .

FIG. 6, unstained free-hand section of a telium of *Pucciniostele hashikoi* (Hirats. f.) Cum. on *Ampelopsis* sp. (from Cheo 2939), 7, a single teliospore of *Pucciniostele hashikoi* showing the outer wall swollen over the germ pores; 8, teliospore of *Phragmidium griseum* Diet. on *Rubus* sp. (from Cheo 1123); 9, teliospore of *Phragmidium rubi-thunbergii* Kusano on *Rubus* sp. (from Cheo 23); 10, teliospores of *Phragmidium rosae-dahuricae* Miura on *Rosa* sp. (from Cheo 1381).  $\times 800$ .

## NOTES AND BRIEF ARTICLES

MUSHROOMS IN THEIR NATURAL HABITATS, by Alexander H. Smith. Two volumes: Vol. I, Text (I-XIV + 626 pp.) with eleven text-figures (line-drawings); Vol. II, Illustrations (Stereophotographs by William B. Gruber). Published in 1949 by Sawyers, Inc., Portland, Oregon (Price \$26.50).

This is a new and a successful approach to the study of mushrooms and their relatives. The work is distinguished by the fact that it combines natural-habitat color stereo-photographs with technical descriptions. It is clearly an attempt to improve on the art of presenting to the public authentic and interesting information on the agarics and other fleshy forms in both the ascomycetes and basidiomycetes. The attainment of this goal is facilitated by the author's readable style. The volumes are not designed primarily for the specialist but rather for those of less experience. But the book promises to assist in the identification of certain fleshy fungi, to stimulate an interest in them, and to help one gain a basic understanding of this engaging group of organisms.

Volume I is text; volume II is really a plush-lined box containing a Sawyer's view-master and an album of 33 reels of color photographs, made in the field and woods. As for the text (Volume I), about two-thirds of its pages are devoted to the salient facts relative to each species included, embracing synonymy and a technical description, and a discussion of edibility, habit, habitat, and distribution. Several keys to groups, genera, and species are included. The section on agarics constitutes more than half the text.

There are also many pages of fresh discussion bearing on the subjects of collecting, techniques for microscopic study, nomenclature, and mycophagy; some five pages of literature; a glossary of more than six hundred items; and an adequate index.

Mycologists, especially agaricologists, will welcome the chapter entitled: "Laboratory Techniques and the Study of Microscopic

Characters of Importance in the Classification of Fleshy Fungi." In that section of some forty-five pages, Dr. Smith describes the use of chemical reagents, the microscopic characters of agarics including his morphological grouping of spores, the technique of fruit-body study, and a good discussion of the "tissues,"—hymenium, gill trama, pileus, and stipe. The principal microscopic structures are illustrated by line-drawings in eleven text-figures.

As the author has proceeded in his agaric studies over the last twenty years, he has met with hundreds of species and has felt the inadequacy of the prevailing methods and techniques for distinguishing species. Mere morphological descriptions of the gross features along with spore-dimensions have proved to him and us insufficient to separate certain species which are obviously distinct. He has, in recent years, been willing, if not constrained, to extend inquiry further into the details of microscopic structure of the pileus, stipe, hymenium, and gill trama in an effort to bring all usable characters into play for taxonomic purposes. He has also, in recent years, investigated and extended the use of chemical reagents in an attempt to discover the clincher in a taxonomic problem. His experience in these matters shows up throughout the text, and is clearly suggestive of new approaches to a more satisfactory agaric taxonomy.

Thus, one is impressed with the new features of the two volumes: the subject is treated in accordance with modern research methods and concepts; and the illustrations are stereo-photographs in natural color (mostly excellent!) and habitat. Moreover, the descriptions in the text were drawn chiefly from the particular specimens illustrated. The collections, of course, have been preserved, and are on deposit in the University of Michigan Herbarium.

The author treats 230 species, the selection of which was determined by a number of considerations: those species common to the Northern United States and Southern California (the war curtailed efforts to get photographs in the Southeast and in the Gulf States); special effort was made to include endemics; representatives of as many genera as possible are included; both edible and poisonous species are represented; and finally, no species is treated unless a suitable natural-habitat photograph could be secured.

Wherever justified, in the opinion of the author, he breaks away from conventional classification and nomenclature. Thus, he uses XEROMPHALINA, based on *Omphalia campanella* Quélet; OMPHALINA, including only the section Collybiariae of *Omphalia*; CATHELASMA, including species of *Armillaria* exhibiting bilateral gill trama and amyloid spores; CYSTODERMA, embracing those species of *Lepiota* (and *Armillaria*) with a granulose covering of the pileus and lower portion of the stipe; LEUCOPAXILLUS, comprising certain species of *Clitocybe*, *Tricholoma*, and *Pleurotus* with amyloid spores; LYOPHYLLUM, based on certain species of *Collybia*, *Mycena*, *Clitocybe*, and *Tricholoma* which exhibit small bodies in the basidia or base of the sterigmata which stain very dark in ferric aceto-carminic; CONOCYBE, species of *Galera* with a cellular to hymeniform cuticle of the pileus; ROZITES, a *Cortinarius* with a rudimentary volva and a membranous partial veil; GYMNOPIUS, "a logical aggregation of species formerly grouped in *Pholiota*, *Flammula*, and *Naucoria*"; NAEMATOLOMA, fleshy species of *Hypopholoma*; PSEUDOCOPRINUS, *Coprinus*-like agarics, the gills of which are non-deliquescent; and also LACCARIA, VAGINATA, LIMACELLA, RHODOPHYLLUS, and GALERINA. The genus CANTHARELLUS is placed in the family Cantharellaceae, along with *Craterellus*, *Leptotus*, and *Arrhenia*.

The author reports one new species, **Cystoderma Gruberianum**, on page 363 of the text; and one new variety, **Lepiota Molybdites** (G. Meyer ex Fr.) Sacc. var. **marginata**, on page 429. Latin descriptions are provided—to the joy of several, and perhaps to the displeasure of the non-conformists.

The International Rules of Botanical Nomenclature (1930) are followed, in order, as Smith says, to give validity and stability to naming.

Those who have followed the fortunes of the author of this book will be pleased with this his most recent effort. It is a novel two-volume publication, and precisely hits the intended mark. The work is indeed excellent, one which will prove unusually valuable for all who would know the mushrooms and other fungi.—L. R. HESLER.

DIE PILZE, Grundzüge ihrer Entwicklungsgeschichte und Morphologie by Dr. Ernst Gaüman. Lehrbücher und Monographien aus dem Gebiete der Exakten Wissenschaften n. 19. E. Birkhauser & Cie. Elisabethen Strasse 15, Basel, Switzerland. Pp. 1-382. 440 figs. 1949.

All mycologists will welcome this revised treatment on the developmental history and morphology of the fungi by the noted Swiss mycologist. The text, in German, is distinguished by its clarity and preciseness of statement. A remarkable feature is that the author has treated his subject in 382 pages replete with illustrations. The brevity of the treatment of many groups will probably be deplored by some specialists, but one seeking an over all treatment of the fungi will appreciate the evenness of the treatment. The devotion of a little over a page to the whole group of Fungi Imperfecti is in keeping with the author's thesis that when the fungi have all been arranged into a natural system there should be nothing left in the Imperfecti.

In Gaüman's scheme of the evolution of the fungi, see p. 10, the Siphonales and Flagellatae are the two points of origin of the fungi. The line from the Siphonales leads to the Oomycetes where it ends.

The Archimycetes, derived from the Flagellatae, are characterized by lacking a cell wall, living parasitically in the cells of the host, and by the thallus being holocarpic. They are considered to be an endpoint of development at a level below that of the Phycomycetes.

The main line of development leads through the Chytridiales from the Flagellatae. At the Chytridiales it divides into three branches, all of equal level. These are the Monoblepharidales, Blastocladales, and Zygomycetes. These in addition to the Oomycetes, constitute the group known as the Phycomycetes. All are characterized by a cell wall.

The Ascomycetes are derived from the Phycomycetes through the Zygomycetes. The two most primitive groups are the Taphrinales and Endomycetales. The Taphrinales represent an endpoint of development whereas the Plectascales are derived through the Endomycetales and represent a higher level of development. From

the Plectascales numerous branches lead to the various well known orders of Ascomycetes, including the Clavicipitales. The Tuberales are derived from the Pezizales, and constitute the most highly developed group of Ascomycetes.

The Basidiomycetes are derived from the Ascomycetes from the vicinity of the Pezizales and Helotiales. The Gasteromycetes are derived from the Corticiaceae in the Hymenomycetes. The Auriculariales, Tremellales, Hymenomycetes and Gastromycetes are represented as of approximately comparable levels of development with the Ustilaginales and Uredinales as the most advanced Basidiomycetes, and derived from the Auriculariales.

Whether one agrees with this scheme in its entirety or not, it must be admitted that the author has handled his material well and produced a well balanced treatise which will always be an excellent point of departure for a detailed discussion of individual orders and families.—ALEXANDER H. SMITH.

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A note from Dr. Bisby states that the third edition of "A Dictionary of the Fungi" has been issued. The price is three dollars (\$3.00). It can be obtained from the Imperial Mycological Institute, Kew, Surrey, England.—A. H. SMITH.

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A correction: In the article on "Pullularia," p. 436, in lines 8-10, the letters A and B were reversed. A is the panel showing mold growth whereas B is clear of growth.—ERNEST S. REYNOLDS.

## MYCOLOGIA

## FINANCIAL STATEMENT

(July 1, 1949-June 30, 1950)

<i>Current receipts (joint funds):</i>	
Mycological Society (member's subscriptions) . . .	\$2,048.00
Subscriptions . . . . .	3,742.82
Sale of back sets (vol. 25 and later) . . . . .	382.63
Payment for excess pages . . . . .	883.10
	<hr/>
	\$7,056.55
<i>Special funds:</i>	
Sale of back sets (vol. 1-24) . . . . .	\$ 176.50
Interest on endowment . . . . .	635.00
	<hr/>
	\$ 811.50
Total income . . . . .	<hr/>
	\$ 7,868.05
<i>Cost of printing and distribution:</i>	
Printing, binding, mailing . . . . .	\$6,315.94
Engraving . . . . .	1,479.17
	<hr/>
	\$7,795.11
<i>Miscellaneous expense:</i>	
Adjustment—July 1, 1950 . . . . .	\$ 11.22
Office expense . . . . .	162.44
	<hr/>
Total expense . . . . .	\$ 7,968.77
	<hr/>
<i>Excess of expense over income</i> . . . . .	(100.72)
Unexpended reserve July 1, 1949 . . . . .	\$4,716.23
Adjustment—July 1, 1949 . . . . .	33.97
	<hr/>
	\$ 4,750.20
	<hr/>
Unexpended reserve, June 30, 1950 . . . . .	\$ 4,649.48
Endowment fund . . . . .	14,000.00
	<hr/>
Total . . . . .	\$18,649.48

The above MYCOLOGIA funds are administered by the New York Botanical Garden, and the balances at June 30, 1950, are in agreement with the amounts shown in the financial statements of that organization which have been examined by Price, Waterhouse & Co.

DONALD P. ROGERS,  
Managing Editor

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\* This index prepared by Henry A. Imshaug.

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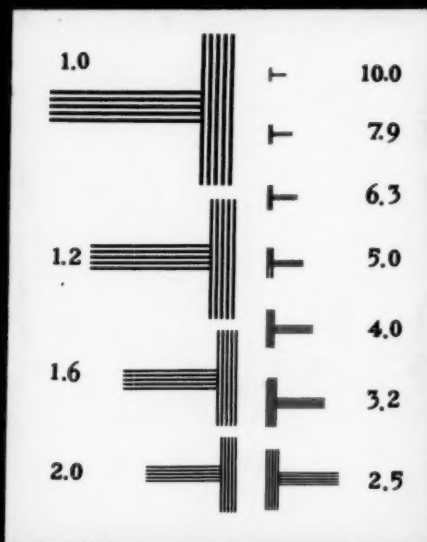
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